Hypocholesterolemic Effect of Ethanol Extract of Ananas comosas (L.) Merr. Leaves in High Cholesterol Fed Albino Rats

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ABSTRACT

The hypocholesterolemic effects of the ethanol extract of Ananas comosas (L.) Merr. (Pineapple) was investigated in 5 groups of albino rats, 7 in each group. Group I rat received only distilled water and served as normal control. Groups II, III, IV and V were made hypercholesterolemic by feeding cholesterol orally suspended in 2% cholic acid mixed soybean oil (1 ml/kg bw) at a dose of 100 mg/kg bw daily up to 60 days. Group II rat received 1 ml distilled water while animals of Group III, IV and V received ethanol extract of Ananas comosas (L) Merr. at the dose of 1000 mg/kg, 500 mg/kg and 100 mg/kg body weight respectively daily up to 60 days in addition to cholesterol as above. At the end of 60th day animals were sacrificed and blood samples were collected for measurement of lipid profiles using enzymatic kit. Administration of cholesterol caused a significant rise (p<0.001) in the serum levels of total cholesterol, LDL-cholesterol and triglycerides. Simultaneous administration of three different doses of Ananas comosas (L) Merr. extract namely 1000 mg/kg, 500 mg/kg and 100 mg/kg for 60 days decreased serum cholesterol level by 62%, 47%, and 42% respectively (p<0.001); serum triglycerides level by 62.7%, 58.7%, and 49.09% respectively (p<0.001); serum LDL level by 70.5%, 53.9% and 51.15%, respectively (p<0.01). However, serum HDL level was not affected significantly. The cumulative results clearly indicate ethanol extract of Ananas comosas (L) Merr. leaves possesses potent hypocholesterolemic effect.

Key words: Hypocholesterolemic effect; Ananas comosas (L.) Merr.

INTRODUCTION

Hypercholesterolemia or Hyperlipidemia is one of the greatest risk factors contributing to prevalence and severity of cardiovascular diseases. The epidemiological data reported that almost 12 million people die of cardiovascular diseases and cerebral apoplexy each year all over the world. Therefore, it is very important to pay attention to early stage prevention and control of hyperlipidemia in a comprehensive way. Treatment of hyperlipidemia involves diet control, exercise, and the use of lipid-lowering diets and drugs. However, some patients cannot tolerate the adverse effects of these oral drugs (Sacco 2001, Stone 1996). As a consequence, there continues to be a high demand for new oral antihyperlipidemic drugs.

Management of hyperlipidemia without any side effects is still a challenge to the medical system. Plant products are frequently considered to be less toxic and freer from side effects than synthetic ones. Plants play a major role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycemics, and hypolipidemics.

Ananas comosas (L) Merr. (Family: Bromeliaceae), also named Pineapple has long been one of the most popular of tropical and subtropical fruits. It is grown extensively in the hilly area especially in Chittagong hill tracts of Bangladesh. Besides agricultural utilities such as being a fruit with nutritional value, some folk medicinal uses have been found. The cortex of the plant has been using as the medication in dysuria (Weggemans and Trautwein 2003), auxipharic, antitussive and anti diarrheal agents. Leaves has been using as antidyspepsia (Weggemans and Trautwein 2003). Indigenous people and traditional medicine practitioners of Bangladesh use the leaves of this plant as anthelmintic and in order to reduce the different symptoms of CVD. But no scientific data still exists to support this claim. In this perspective, the present
study was designed to assess the hypolipidemic effect of *Ananas comosus* (L.) Merr. ethanol extract in high cholesterol fed albino rats.

**Materials and Methods**

**Collection of Plant material**

*Ananas comosus* (L.) Merr. leaves were collected from Chittagong Hill Tracts (CHT), Bangladesh. The plant was taxonomically identified by Dr. Md. Yusuf, Ex-Chief Scientific Officer, BCSIR Laboratories Chittagong and a voucher specimen has been deposited in the Herbarium of BCSIR laboratories, Chittagong.

**Preparation of ethanol extract**

The collected leaves of *Ananas comosus* (L.) Merr. were cut into small pieces, air dried at room temperature for about 10 days and ground into powder form and stored in an airtight container. 500 gm of powder was then macerated in 90% ethanol for 7 days at room temperature with occasional stirring. The ethanol extract of the plant was collected in a separate container and concentrated under reduced pressure below 50 °C through rotatory vacuum evaporator. The concentrated extracts (6.5%) were air dried at room temperature and finally stored in the refrigerator at 4°C.

**Animals and Diets**

Wistar albino rats of the either sex weighing between 100-110 g obtained from animal house of BCSIR laboratories, Chittagong were used for the present study. The animals were acclimatized to room temperature (28±5°C) with a relative humidity of 55±5 % in a standard wire meshed plastic cages for 4 to 5 days prior to commencement of the experiment. During the entire period of study the animals were supplied with standard pellet diet and water *ad libitum*.

**Chemicals**

Cholesterol and cholic acid were purchased from Loba Chemie Pvt Ltd, Bombay and Kits for all the parameters of lipid profile were purchased from Human, Germany.

![Fig 1. Experimental design and work flow](https://example.com/fig1)

**Test groups**

<table>
<thead>
<tr>
<th>Group I (Normal control)</th>
<th>Group II (Hypercholesterolemic control)</th>
<th>Fed with</th>
</tr>
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<tbody>
<tr>
<td>1 ml distilled water</td>
<td>100 gm/kg body weight of cholesterol</td>
<td></td>
</tr>
<tr>
<td></td>
<td>1 g/kg weight of AC extract and 100 gm/kg body weight of cholesterol.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0.5 g/kg weight of AC extract and 100 gm/kg body weight of cholesterol.</td>
<td></td>
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<tr>
<td></td>
<td>0.1 g/kg weight of AC extract and 100 gm/kg body weight of cholesterol.</td>
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</table>

Animals are sacrificed and blood is collected. TC, TG, HDL and LDL were assayed. The observed data were analyzed statistically which showed that the AC extract significantly reduces the cholesterol. The most successful dose of *A. comosus* (L.) Merr. leaves extracts to lower the TC, TG and LDL level and to increase the HDL level was 500 mg/kg body weight, because our result clearly shows that, it significantly decreases the bad cholesterol (LDL) and increases the good cholesterol (HDL) in case of group IV when the rats were treated with Cholesterol (100 mg/kg) and AC (500 mg/kg) compared with untreated group (Table 1).
Assessment of hypocholesterolemic activity

The effect of *A. comosus* (L.) Merr. ethanol extract on lipid profile was evaluated by cholesterol induced hypercholesterolemia as described by (Shukla *et al.* 1995). Thirty five albino rats were divided into following five groups, where 7 rats were taken in each group.

Group I: Normal rat fed with normal basal diet and orally administered 1ml distilled water daily up to 60 days.

Group II: Hypercholesterolemic rats administered 1ml distilled water daily up to 60 days.

Group III, IV and V: Hypercholesterolemic rats orally administered *A. comosus* (L.) Merr. ethanol extract at the dose of 1000 mg/kg, 500 mg/kg and 100 mg/kg body weight respectively daily up to 60 days.

Hypercholesterolemia was induced in animals of group II, III, IV and V by giving orally cholesterol 100 gm/kg body weight by gastric feeding needle, once a day, suspended in 1 ml/kg body weight of 0.2% cholic acid mixed soyabean oil for sixty days. Water and food were given *ad libitum* to animals of all the groups for the entire period study.

Analytical

At the 60th day the animals were fasted overnight and sacrificed for blood collection. The blood was collected from the heart of the rats under light diethyl ether anesthesia. The blood was immediately centrifuged (2500 rpm, 4°C) and the serum was collected for biochemical analysis and kept at -20°C until the lipoprotein assay. Total Cholesterol (TC), High Density Lipoprotein (HDL), and Triglycerides (TG) were measured by spectrophotometric method using the assay kit supplied by Human, Germany. Low Density Lipoprotein (LDL) was calculated with the Friedewald formula (Friedewald *et al.* 1972).

Acute Toxicity Study

Acute toxicity of ethanol leaves extract of *A. comosus* (L.) Merr. was carried out orally on thirty Wistar albino rats (weighing 150-160 gm) at three different dose levels namely 5 gm/kg, 4 gm/kg and 3 gm/kg body weight for 14 days. Five male and five female rats for each dose were closely observed for 24 hours for any mortality and next ten days for any delayed toxic effect. Their food consumption and growth rate were also examined once daily up to fourteen days.

Statistical Analysis

All the values in the test are expressed as Mean ± SEM [Standard error of the Mean]. Statistical difference between the mean of the various groups were analyzed by Student’s “t” test using the software “Microsoft Excel-2007”. P values <0.05 or less were considered as significant.

Results

Effect of *A. comosus* (L.) Merr. extract on serum lipid profile of albino rats

Table 1 shows the values of serum lipid profile in normal, hypercholesterolemic control and extract treated groups. There was a significant (P<0.001) increase of TC (171.1%), TG (109.2%), LDL (255.7%) and a moderate increase of HDL (20.76%) in hypercholesterolemic control group II in comparison to the normal control group I. When cholesterol-treated group III was administered with the *A. comosus* (L.) Merr. extracts of 1000 mg/kg body weight, a significant (P<0.001) decrease of TC (62.04%), TG (62.75%) and LDL (70.52%) were observed with a slight decrease of HDL (17.6%) in comparison to the hypercholesterolemic control group II. In the cholesterol-fed group IV the treatment of 500 mg/kg weight plant extract also significantly (P<0.1) decrease the TC (47.36%), TG (58.7%) and LDL-cholesterol (70.52%) with a decent increase of HDL (18.00%). The third treatment group that was treated both with cholesterol and 100 mg/kg of *A. comosus* (L.) Merr. extract also experienced a good decrease in TC (42.02%), TG (49.09%) and LDL-cholesterol (51.15%) with slight elevation in HDL levels (5.41%) (Table1).
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Table 1. Effect of ethanol leaves extract of *A. comosus* (L.) Merr. on serum lipid profile of albino rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Total Cholesterol (mg/dl)</th>
<th>Triglyceride (mg/dl)</th>
<th>HDL-Cholesterol (mg/dl)</th>
<th>LDL-Cholesterol (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Distilled water (1 ml)</td>
<td>61.74 ± 3.57</td>
<td>64.31±3.57</td>
<td>14.21±1.26</td>
<td>34.67±4.09</td>
</tr>
<tr>
<td>II</td>
<td>Cholesterol (100 mg/kg) &amp; Distilled water (2 ml)</td>
<td>167.38±5.2</td>
<td>134.54±3.63</td>
<td>17.16±1.03</td>
<td>123.31±5.19</td>
</tr>
<tr>
<td>III</td>
<td>Cholesterol (100 mg/kg) &amp; AC (1000 mg/kg)</td>
<td>63.50±3.22</td>
<td>50.11±4.45</td>
<td>14.14±1.31</td>
<td>36.34±2.39</td>
</tr>
<tr>
<td>IV</td>
<td>Cholesterol (100 mg/kg) &amp; AC (500 mg/kg)</td>
<td>88.10±5.07</td>
<td>55.54±5.63</td>
<td>20.25±0.84</td>
<td>56.74±5.09</td>
</tr>
<tr>
<td>V</td>
<td>Cholesterol (100 mg/kg) &amp; AC (100 mg/kg)</td>
<td>92.02±4.18</td>
<td>68.49±9.09</td>
<td>18.09±1.76</td>
<td>60.23±5.83</td>
</tr>
</tbody>
</table>

Here values are expressed as MEAN±SEM and number of rats in each group=7. % activities are shown within parentheses. ↑= increase, ↓= decrease. AC= *Ananas comosus* (L.) Merr. Leaf extract

P Values (Student’s t-test):

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<tr>
<th>Group B vs Group A</th>
<th>Group C vs Group A</th>
<th>Group D vs Group B</th>
<th>Group E vs Group B</th>
<th>Group F vs Group B</th>
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<tbody>
<tr>
<td><strong>P&lt;0.001</strong></td>
<td>*** P&lt;0.001**</td>
<td>** P&lt;0.01**</td>
<td>** P&lt;0.05**</td>
<td>NS ~ Not Significant</td>
</tr>
</tbody>
</table>

% activity:

<table>
<thead>
<tr>
<th>Group B compared with Group A</th>
<th>Group C compared with Group A</th>
<th>Group D compared with Group B</th>
<th>Group E compared with Group B</th>
<th>Group F compared with Group B</th>
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Acute toxicity test

In acute toxicity study, oral administration of ethanol leaves extract of *A. comosus* (L.) Merr. did not produce any mortality in rats up to the highest dose of 5 gm/kg body weight. Animals also did not show any stereotypical symptoms associated with toxicity, such as Inapetence, tremor, ataxy, diarrhoea or increased diuresis. Behavior of all treated animals appeared normal and no significant change in the body weight as well as their daily food consumption were observed. Hence, the extract was considered to be without gross or acute toxic effects on rats (Table 2).

Discussion

It is well documented that elevated total cholesterol and low density lipoprotein cholesterol (LDL) levels promote atherosclerosis and cardiovascular complications (Dominiczak 1998). The agents that can lower serum cholesterol have gained wide therapeutic value. *Ananas comosus* (L.) Merr. has been reported to have a variety of biological effects including antidiuresia, antiauxipharic, antitussive and antiquirare agents. Leaves has been using as antidyspepsia and hypolipidaemic activity. For the hypolipidaemic effects, scientific data on its efficacy is rare. In the present study, we examined whether the *A. comosus* (L.) Merr. leaves extracts is capable to improve the lipoprotein profile in rats. It has been reported that hyperlipidaemia (increased level of TG, TC and LDL-C) is an important risk factor for development and progression of CHD (Davigon and Cohn 1996; Libby et al 2000 and Brbiller et al 1996).

In the present study, a severe degree of hypercholesterolemia was induced in group II to V rats by feeding cholesterol (100 mg/kg bw/day) orally once daily for 60 days. Group II animals which served as untreated controls continued to receive the same amount of cholesterol for four more weeks, in order to ensure hypercholesterolemia. As it is well known that, serum cholesterol level would fall down even without treatment if cholesterol administration was discontinued. Simultaneous administration of different doses (1000 mg/kg, 500 mg/kg and 100 mg/kg) of both ethanol extract and cholesterol in group III, IV and V (treated) ensured that any fall in serum cholesterol was due to drug treatment but not due to cessation of cholesterol intake in diet.

Our results revealed that the treatment with the *A. comosus* (L.) Merr. leaves extracts at the dose of 1000 mg/kg body weight could successfully and
significantly (P<0.001) decrease the TC, TG and LDL level but could not raise the HDL level (Table1). Therefore, this dose may not be an ideal one to improve the lipoprotein parameter. The most successful dose of A. comosus (L.) Merr. leaves extract to lower the TC, TG and LDL level and to increase the HDL level was 500 mg/kg body weight, because our result clearly shows that, it significantly decreases the bad cholesterol (LDL) and increases the good cholesterol (HDL) in case of group IV when the rats were treated with Cholesterol (100 mg/kg) and AC (500 mg/kg) compared with untreated group. Administration of lower doses of AC (100 mg/kg) in hypercholesterolemic rats also brought down the TC, TG and LDL level in comparison to untreated group and elevated the HDL level slightly.

A logical therapeutic strategy to prevent or treat atherosclerosis and reduce the incidence of CHD events is to target the hyperlipidaemia by lipid-lowering drugs with HDL-increasing activity. Several researches have documented the role of phytonutreints such as flavonoid in this regard (Weggemans and Trautwein 2003). The leaves of Ananas comosas (L.) Merr. were reported to have many phytochemicals specially the fibers such as hemicellulose. They were also reported to have many soluble fibers like oat, pectin etc. (Umashankar 1981). The soluble fibers on the contrary were reported to lower the cholesterol level (Lisa et al 1999). Considering these facts, it may be possible that these active principles are responsible for lowering TC and LDL-C and elevating HDL-C in group III rats.

Weidong et al showed that, after 15 days of treatment of diabetic and hypercholesterolemic rat ethanolic extract of Ananas comosas (L.) Merr. leaves significantly decrease TG (-5.01%, P<0.01), TC (-23.3%, P<0.01), LDL-c (-47.9%, P<0.01) and significantly increased HDL-c levels (66.2%, P<0.01). In this study we have found that after 60 days of treatment of the hypercholesterolemic rats, ethanolic extract of Ananas comosas (L.) Merr. leaves (1000 mg/kg body weight of rat) significantly decrease TG (-62.75%, P<0.01), TC (-62.06%, P<0.01), LDL-c (-70.52%, P<0.01) and HDL-c (-17.6%, P<0.01). In both cases TC, TG and LDL-c levels were reduced. But in the previous experiment showed the increase of HDL-c levels where the present study showed its decline in case of using 1000 mg/kg body weight of AC extract (Weidong et al 2005).

The effect of 500 mg/kg body weight of leave extract has been shown to lessen TC, TG and LDL-c level and increase HDL-c level which is very much similar to the previous data (Weidong et al 2005). The 500 mg/kg body weight of the leave extract of Ananas comosas (L.) Merr. significantly decrease TG (-58.7%, P<0.01), TC (-47.36%, P<0.01), LDL-c (-53.98%, P<0.01) and significantly increased HDL-c levels (+18.0%, P<0.01).

The 100 mg/kg body weight of the leaves extract of Ananas comosas (L.) Merr. significantly decrease TG (-49.09%, P<0.01), TC (-42.02%, P<0.01), LDL-c (-51.15%, P<0.01) and significantly increased HDL-c levels (+5.41%, P<0.01). This effect of the AC extract on lipid profile is poorly supported by the previous data (Weidong et al 2005).

The results of the present study revealed that the ethanol leaves extract of A. comosus (L.) Merr. possess significant hypocholesterolemic activity. The acute toxicity studies also showed that the extract has a high safety profile as neither death nor symptoms associated with toxicity was observed at high dose level (5 gm/kg) in rats. Since crude ethanol extracts of A. comosus (L.) Merr. showed significant hypocholesterolemic properties, we assume that different active secondary metabolites are present in crude extracts and perhaps some of these compounds may operate in a synergistic manner. However,
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Further studies are necessary to elucidate the mechanism behind this effect. This report may serve as a footstep on this aspect.

Acknowledgement

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REFERENCES


