A comparative study of allitin and garlic on lipid turnover in a teleost, *Anabas testudineus* (Bloch)

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Both allitin and garlic have anti-lipogenic properties substantiated by the activity of three lipogenic enzymes and lipid profiles. The rise in the HDL levels and simultaneous fall in the LDL upon garlic intake is the most convincing indicator of reduced lipid concentration. However, the administration of allitin recorded a decrease in the HDL and LDL levels, but when calculated on a percentage basis, there was a marginal increase in the HDL level. On the basis of results, it can be concluded that garlic or its derivatives have hypolipidaemic effect in submammalian vertebrates also. The cholesterol lowering effect of allitin and garlic can be commercially exploited for producing fish with low cholesterol for possible human consumption.

Garlic (*Allium sativum*), a member of the Lily family is one of the most widely researched medicinal plants. Its purported health benefits are numerous, including cancer chemopreventive, antibiotic, anti-hypertensive, and cholesterol-lowering properties. The organosulphur compounds present in it are responsible for the various medicinal effects ascribed to this plant. Strangely, the intact bulbs of garlic have very little medicinal significance. One of the major components of freshly crushed garlic homogenates is an odoriferous compound named allilin. It is formed by the action of the enzyme allilase on allin, the major cysteine sulphoxide present in garlic. Considerable variations have been reported in the quantity of organosulphur compounds present in fresh and commercially available garlic products.

Allitin is an analogue of allilin. It contains 98% diallyl disulphide (CH\(_2\)=CH-CH\(_2\)-S-S-CH\(_2\)-CH=CH\(_2\)) and 2% diallyl trisulphide (CH\(_2\)=CH-CH\(_2\)-S-S-S-CH\(_2\)-CH=CH\(_2\)). It is prepared synthetically from allyl thiol by oxidation with iodine. It is purified by column chromatography and vacuum distillation.

Plasma and liver cholesterol were assayed because they belong to the fast turnover cholesterol pool in the human body. Plasma cholesterol levels and coronary heart disease being so closely linked and have drawn the attention of researchers to focus their study on changes of plasma cholesterol levels. The cardioprotective effects of garlic are more due to its cholesterol lowering effect. Warshafsky et al., in a study on human subjects have reported that consumption of 900 mg of garlic per day could decrease the serum cholesterol by over 9%. In almost a similar study by Silaghi and Neil, eight hundred mg garlic per day reduced the serum cholesterol by almost 12%. However, Isaacsohn et al., contradicted this by their assessment that even 12 weeks of garlic administration was ineffective in lowering cholesterol levels in human subjects. Recently, Dhuley et al. have reported the efficacy of diallyl disulphide as a hypoglycemic and antioxidant agent in rats.

The present study examined the ability of allitin, an analogue of allilin and raw garlic to exert an anti-lipogenic activity in a teleost, *Anabas testudineus* (Bloch). Although similar experiments conducted on rat and mice have been reported, knowledge on the lipid turnover in a lower vertebrate is still scanty. The present work is subdivided into two sections, the first with allitin and the second with feeding raw garlic. In the first section fish was administered with allitin intraperitoneally at a concentration of 10 mg/kg body weight for 10, 20 and 30 days using physiological saline as vehicle. In the second section, raw garlic was given in conjunction with the standard fish feed at concentrations equivalent to human consumption of 10, 20 and 40 g garlic/day for 45 days. The controls were given standard fish feed.
Materials and Methods

Section I:

*Anabas testudineus* (Bloch) captured from the Kananamula canal running through the outskirts of Thrivanthapuram City (Kerala State) were captured using a net and transferred to cement tanks with proper aeration and natural light. Healthy adult fish (60 ± 5 g) after acclimatization for a month were separated into six groups of 10 each in similar sized glass tanks, each three each serving as control and experimental. The tanks were placed in the laboratory at suitable locations in order to get sufficient light and aerators were used for air supply. Feed (high fat feed) prepared by adding 20 g beef fat to 80 g the standard 40% protein feed (prepared as per Hardy 11) pelletized and sundried was given *ad libitum*. Water in the tanks was changed daily to avoid turbidity.

Allitin used was prepared by the alkaline hydrolysis of S-allyl-thiouronium chloride under inert (nitrogen) atmosphere, which yielded allyl thiol. Oxidation of this thiol with iodine gave crude diallyl disulphide, which was purified by column chromatography (SiO₂; elution with petroleum ether, 40°-60°C) and vacuum distillation (bp. 82°C/13 mm). The colourless product obtained contained 98% diallyl disulphide and approximately 2% diallyl trisulphide as estimated by GLC and high pressure liquid chromatography (Micro Bonda Pack 75% aqueous methanol 12). This was the sample, given the trivial name allitin, used in the present studies.

The experimental animals were administered intraperitoneally with allitin at a dose of 10 mg/kg body weight for a period of 10, 20 and 30 days using physiological saline as the vehicle. The controls were given vehicle only. At the end of the stipulated period and overnight fasting, blood was collected in heparinized tubes by puncturing the heart. The plasma separated by centrifugation was collected in eppendorf tubes and kept at -4°C for total cholesterol, HDL and LDL determination 13, employing chod/pod phosphotungstate method. The fish were then sacrificed by spinal transection and the liver was separated, washed in saline, blotted and used immediately for lipogenic enzymes assay that included malic enzyme (EC.1.1.1.40) 14, glucose -6- phosphate dehydrogenase (EC.1.1.1.49) 15, and isocitrate dehydrogenase (EC.1.1.1.42) 16. Total lipid 17 and its profile (tissue cholesterol 18, triglycerides (modified) 19, free fatty acids 20 and phospholipids 21) were also estimated.

Section II:

After acclimatization similar to section I, the fish (60 ± 5 g) were segregated into four tanks of 10 each. While those in tank one served as the control, the remaining three as experimental groups. All physical parameters were the same as in section I. Feeding and change of water were done every morning at 10 hrs. While the control group was given food as in section I, the experimental groups were provided with the same type of food having additionally crushed raw garlic added to it in concentrations equivalent to human consumption of 10, 20 and 40 g/day. The amount of garlic required to equalize the stipulated human consumption through food intake was calculated after assessing the amount of food consumed by the fish. One gram of feed consumed/fish/day (established after study) contained 0.017 g garlic to equalize 20 g garlic consumed by a person weighing 70 kg. Both control and experimental groups consumed the same quantity and were given food *ad libitum*. The time of sacrifice was so adjusted that the duration of the experiment was 45 days. Blood and various tissues were collected for different estimations after overnight fasting. Blood collected in heparinized tubes by direct puncture into the heart was centrifuged and the plasma separated was transferred into eppendorf tubes and kept at -4°C for further study of cholesterol, HDL, LDL, and triglycerides. The liver and muscle tissue excised immediately after spinal transection were washed in saline, blotted and frozen for the study of total lipid.

Chemicals used in both sections were of the analytical grade procured from SRL, Bombay. The data were analysed using Student’s t test for the first section and Duncan’s multiple range test for the second section.

Results and Discussion

The results are presented in Tables 1 and 2 (section 1) and Table 3 (section II).

An overall evaluation of the results confirm the lipid lowering properties of garlic and/or its derivatives, especially diallyl disulphide (DADS), the major constituent of allitin, for the first time in a teleost. Allitin and raw garlic are undoubtedly very effective agents in lowering tissue (hepatic and muscle) and plasma lipids in higher animals and it has been demonstrated in many experimental animals 22. An analysis of the effect of garlic showed a significant reduction of serum total cholesterol 7. Liver being the most important drug-metabolizing organ was used to estimate...
the various lipid parameters\(^2\). The results obtained from this study confirm a decrease in the activity of lipogenic enzymes after allitin administration. Various other reports confirm the decreased activity of lipogenic enzymes after allitin administration, which in turn plays a very important role in lowering the lipid turnover in animals. Studies conducted in rats have ascertained that allitin (100 mg/kg body weight) reduced plasma cholesterol significantly 30 min and 1 hr after treatment without any change in hepatic cholesterol\(^2\). Garlic when given through diet reduced the cholesterol levels in present experimental fish. The consumption of garlic tends to show a decrease in the plasma cholesterol and LDL level with the maximum effect at the 40 g dose per day. Another finding with allitin was the reduction in both HDL and LDL levels, however, there was a marked increase in HDL (65% and 64%) and decrease in LDL (35% and 36%) at 10 and 20 day doses when compared to their respective controls (55 and 61% for HDL and 45 and 39% for LDL). With the supplementation of raw garlic, HDL level showed a significant increase with a corresponding decrease in LDL. The 40 g garlic administration dose showed a promising result with low plasma cholesterol and high HDL levels. The probable explanation for the reduction in the hepatic total lipid along with cholesterol in fish administered with allitin at all the three doses (10 mg/kg, body weight/day for 10, 20 and 30 days respectively) may be due to the deposition of lipids in other tissues or the conversion to other sources of stored forms in the animal body. The reduction in the lipid profiles substantiated the decrease in the total hepatic lipid level. The total lipid content in garlic fed fish confirmed an inverse relationship between the liver and muscle. Contradictory to the influence of allitin where there was a direct relationship between the liver lipid and blood cholesterol, feeding the fish with garlic

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**Table 1** — Inhibition of lipogenic enzymes in liver of *A. testudineus* following 10 mg/kg body weight/day allitin administration for various durations

<table>
<thead>
<tr>
<th></th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>ME</td>
<td>0.05±0.04</td>
<td>0.05±0.04</td>
</tr>
<tr>
<td></td>
<td>G6PDH</td>
<td>1.20±0.20</td>
<td>1.20±0.20</td>
</tr>
<tr>
<td></td>
<td>ICDH</td>
<td>0.90±0.1</td>
<td>0.90±0.1</td>
</tr>
<tr>
<td><strong>Experiment</strong></td>
<td>ME</td>
<td>0.37±0.01</td>
<td>0.43±0.04</td>
</tr>
<tr>
<td></td>
<td>G6PDH</td>
<td>0.80±0.20</td>
<td>1.0±0.10</td>
</tr>
<tr>
<td></td>
<td>ICDH</td>
<td>0.70±0.20</td>
<td>0.70±0.30</td>
</tr>
</tbody>
</table>

**Table 2** — Inhibition of liver and serum lipid synthesis in *A. testudineus* following 10 mg/kg body weight/day allitin administration for various durations

<table>
<thead>
<tr>
<th></th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>60.19±1.73</td>
<td>52.42±1.11</td>
<td>52.78±0.15</td>
</tr>
<tr>
<td></td>
<td>34±1.0</td>
<td>37.33±0.52</td>
<td>10.40±0.10</td>
</tr>
<tr>
<td></td>
<td>0.70±0.01</td>
<td>0.70±0.02</td>
<td>0.70±0.03</td>
</tr>
<tr>
<td></td>
<td>8.14±0.15</td>
<td>6.67±0.14</td>
<td>6.93±0.07</td>
</tr>
<tr>
<td></td>
<td>2.45±0.04</td>
<td>2.17±0.04</td>
<td>1.83±0.06</td>
</tr>
<tr>
<td></td>
<td>188.89±1.50</td>
<td>135.45±1.79</td>
<td>142.5±2.58</td>
</tr>
<tr>
<td><strong>Experiment</strong></td>
<td>103.97±1.22</td>
<td>89.79±1.10</td>
<td>92.1±1.6</td>
</tr>
<tr>
<td></td>
<td>125±3.75</td>
<td>125±3.75</td>
<td>125±3.75</td>
</tr>
<tr>
<td></td>
<td>202.3±2.4</td>
<td>202.3±2.4</td>
<td>202.3±2.4</td>
</tr>
<tr>
<td></td>
<td>1.93±0.06</td>
<td>1.58±0.02</td>
<td>1.29±0.01</td>
</tr>
<tr>
<td></td>
<td>234.93±2.09</td>
<td>179.89±2.73</td>
<td>179.89±2.73</td>
</tr>
</tbody>
</table>

**Table 3** — Inhibition of liver and serum lipid synthesis in *A. testudineus* following 10 mg/kg body weight/day allitin administration for various durations

<table>
<thead>
<tr>
<th></th>
<th>10 days</th>
<th>20 days</th>
<th>30 days</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Control</strong></td>
<td>84.92±2.20</td>
<td>46.16±1.97</td>
<td>50±2.40</td>
</tr>
<tr>
<td></td>
<td>106.35±2.11</td>
<td>81.61±2.63</td>
<td>81.61±2.63</td>
</tr>
</tbody>
</table>

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\( P \) values: \(< 0.05; ^{1}P < 0.01; ^{2}P < 0.001.\)

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Units: \( \mu g/g; \) \( \mu g/dl \)
increased the hepatic and decreased the muscle total lipid content along with decreased blood cholesterol. So, the reduction in plasma cholesterol may be interpreted on the basis of conversion of serum cholesterol into hepatic lipids. The decrease in the serum LDL levels and total lipid level in muscle tissue encourages using garlic as a therapeutic agent. These findings support the fact that garlic is effective in various ailments of cardiovascular diseases because of its ability to reduce serum cholesterol. Garlic when administered as a daily dose of 2 × 2 capsules (each capsule containing ethyl acetate extract from 1 g peeled and crushed raw garlic) reduced total serum cholesterol, triglycerides and increased HDL-cholesterol and fibrinolytic activity. The results confirm that different garlic-derived organosulfur compounds interfere differently with cholesterol biosynthesis and, thus, may provoke multiple inhibition of the metabolic pathways in response to garlic consumption. Garlic lowers only elevated cholesterol levels. Various garlic extracts exhibited hypocholesterolemic effects on chickens, mainly through the inhibition of the key enzymes in cholesterol and lipid synthesis. The essential oil fraction of both garlic and onion has the same effect. It was therefore concluded that the active principle of garlic and onion is the essential oil, which is a combination of sulphur-containing compound, mainly allyl propyl disulfide and diallyl disulfide. Regular dietary intake of garlic reduces hypertension and hyperlipidemia. Lowering of serum lipids by garlic ingestion may decrease atherosclerosis. Both garlic and onion in the raw state were more effective. Similarly garlic was more potent when compared to onion. Garlic administration to patients with ischemic heart disease (IHD) shows hypocholesterolemic and blood fibrinolytic activity in the initial (4th week) period but never sustained despite its continuous use and returned to the pre-garlic levels by the 12th week. This evidence does not support the popular belief that garlic can bring down cholesterol and after the blood fibrinolytic activity. Small amounts of fresh garlic consumed over a long period of time may be beneficial in the prevention of thrombosis. Garlic as a herbal remedy reduces total and LDL-cholesterol, increases HDL cholesterol, reduces serum TG and lowers arterial blood pressure in patients. These pleiotropic effects of garlic result in a reduction of relative cardiovascular risk for infarction and stroke by more than 50% (ref.38). The present study using garlic in a fish supports this view, barring the non-significant change in the serum TG levels. Garlic (Allium sativum) contains highly active therapeutic principles, which appear to be particularly suitable for prophylaxis of arteriosclerosis. Bordiu et al. reported that the essential oils of onion and garlic can prevent fat-induced hyperlipemia. A marked reduction of serum cholesterol levels (53 and 54%) was observed in rats fed a diet supplemented with 2 or 3% garlic powder. Similar effects of garlic were found in rats' feed containing either cholesterol or lard. Plasma and liver cholesterol as well as total liver lipids were reduced by about 30% by garlic supplementation whereas plasma triacylglycerols were reduced only in the group fed lard. Depressed hepatic cholesterol levels in chickens fed 2% garlic for 14 days were observed by Sklan et al. Various garlic extracts exhibited hypocholesterolemic effects on chickens, mainly through the inhibition of key enzymes in cholesterol and lipid synthesis. Garlic reduces total cholesterol levels in persons whose levels are elevated. The incorporation of garlic in the diet to rats fed with cholesterol had reduced the serum triglycerides and cholesterol levels significantly. Therefore, it may be concluded that allitin, an analogue of allicin and raw garlic demonstrates a cholesterol lowering effect in a fish. This study indicates that raw garlic feeding and

### Table 3 — Influence of dietary garlic on tissue and plasma lipid and its profiles in A. testudineus.

[Values are mean ± SE of 6 animals in each group. Mean values of groups with different superscript letters in a given row are significantly different (P<0.05) as determined by Duncan’s multiple range test]

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>10 g garlic</th>
<th>20 g garlic</th>
<th>40 g garlic</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver lipid</td>
<td>30.65 ± 0.84</td>
<td>45.66 ± 1.0</td>
<td>45.70 ± 0.84</td>
<td>40.15 ± 0.72</td>
</tr>
<tr>
<td>Muscle lipid</td>
<td>15.88 ± 0.37</td>
<td>12.32 ± 0.14</td>
<td>12.41 ± 0.33</td>
<td>13.17 ± 0.45</td>
</tr>
<tr>
<td>Cholesterol (P)</td>
<td>185.18 ± 2.94</td>
<td>188.83 ± 1.47</td>
<td>185.5 ± 3.32</td>
<td>154.32 ± 2.02</td>
</tr>
<tr>
<td>HDL (P)</td>
<td>87.62 ± 0.29</td>
<td>102.5 ± 1.45</td>
<td>113.17 ± 6.97</td>
<td>112.66 ± 1.50</td>
</tr>
<tr>
<td>LDL (P)</td>
<td>97.56 ± 2.66</td>
<td>81.33 ± 2.06</td>
<td>72.33 ± 3.60</td>
<td>41.66 ± 2.29</td>
</tr>
<tr>
<td>TG (P)</td>
<td>35.83 ± 0.96</td>
<td>34.22 ± 0.62</td>
<td>36.77 ± 1.55</td>
<td>37.92 ± 1.00</td>
</tr>
</tbody>
</table>

P = Plasma, HDL = High density Lipid, LDL = Low density lipid, TG = Triglycerides

Units: * : mg/g; † : mg/dl
allitin administration produced a hypocholesterolemic effect as evidenced by changes in the activity of lipogenic enzymes and lipid profile. The decrease in LDL levels and simultaneous increase of HDL when given raw garlic provides evidence for this compound to be used as a therapeutic agent. It will be of interest to include garlic products in aquaculture fish feed and to have fish with low LDL and cholesterol and high HDL, for human consumption.

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References