Anti-hyperlipidaemic effects of fresh and cured Bhallataka Kshaudra (Semecarpus anacardium L.) in animals

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Bhallataka Phala (fruit of Semecarpus anacardium L.), is a well-known anti-hyperlipidaemic drug. Bhallataka is described in all Ayurvedic classics as Rasayana and advocated for various therapeutic purposes such as Kushtha (skin diseases), Arsha (piles), Krimi (worm infestations), Prameha (urine disorders), Medodosha (lipid disorders), etc. Though its anti-hyperlipidaemic activity has been studied; actual differentiation in their therapeutic efficacy due to storage period has yet not been attempted on experimental animals. This prompted to initiate a comparative anti-hyperlipidemic activity of fixed oil (expressed oil) i.e. non-volatile in nature collected from fresh Bhallataka and four months old (cured) Bhallataka samples against cholesterol diet induced hyperlipidaemia in rats. Hyperlipidemia was induced by oral administration of cholesterol (20 % suspension in coconut oil, 5 mL/kg) in morning hours and hydrogenated vegetable oil (5 mL/kg) in evening. The effect of drugs was assessed on body weight, serum biochemical and histological parameters. Both drugs produced significant attenuation of the relative weight of liver in cholesterol-fed animals. Cured sample of Bhallataka provided better effect in lowering serum cholesterol (21.98 %), triglyceride (60.23 %), VLDL (56.82 %); while fresh sample of Bhallataka also found to be effective in lowering serum cholesterol (20.69 %), serum triglyceride (45.59 %), VLDL (46.59 %) in comparison to control group. From the result of the present study, it is concluded that the cured sample of Bhallataka has pronounced anti-hyperlipidemic effect than the fresh sample in experimentally-induced hyperlipidemia in rats.

Keywords: Anti-hyperlipidaemia, Bhallataka, curing, Polyphenolic compounds, Semecarpus anacardium L.

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Introduction

Hyperlipidaemia involves abnormally elevated levels of any one of total cholesterol, low-density lipoprotein, triglycerides or all lipids in the serum blood plasma. The metabolic consequences associated with changes in diet and lifestyle has increased the number of hyperlipidemic individuals who could benefit from lipid-lowering therapy. Lipoproteins play an essential role in the absorption of dietary cholesterol, long chain fatty acids and fat-soluble vitamins so help in transport of triglycerides, cholesterol and fat-soluble vitamins from the liver to peripheral tissues and vice versa. Multiple epidemiologic studies have demonstrated a strong relationship between serum cholesterol, elevated plasma triglyceride levels and coronary heart disease (CHD). Cardiovascular diseases are the number one cause of death globally. An estimated 7.3 million death were due to coronary heart disease and 6.2 million were due to stroke. The traditional system of medicines is a better alternative with minimal side effects to combat these types of disorders.

Bhallataka (Semecarpus anacardium L.) belonging to family Anacardiaceae is a common tree found throughout the country especially in the Himalayas and hotter parts of Indian in dry and moist deciduous forests. The fruit is useful in treating many diseases like Kushtha (skin diseases), Arsha (piles), Krimi (worm infestations), Prameha (urine disorders), Medodosha (lipid disorders) etc and is emphasized as best Rasayana (rejuvenator). Fruit of S. anacardium is well known for its anti-hyperlipidaemic property (Medodoshanashaka). Kala-Prakarsha Samskara (curing) is one of the important Samskara (processing and propagation) especially mentioned in Ayurvedic texts, which plays a major role in propagating the therapeutic efficacy and safety of medicines. Kala Prakarsha Samskara especially emphasizes for Bhallataka i.e. storage of Bhallataka fruits in heap of barley for a period of four months in rainy season. Preservation time alters the drug properties, mainly its

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Several pharmacological and clinical trials are reported pertaining to Bhallataka fruit but experimental studies for comparing the efficacy of fresh and cured samples have not yet been found. Therefore, the present study was planned with an aim to evaluate the comparative anti-hyperlipidaemic efficacy of fresh and cured Bhallataka fruits in albino rats.

Materials and Methods

Drug

Fresh Bhallataka fruits were collected from Jalna (19.83° N 75.88° E) Maharashtra, India during Shucho-Shukre (the month of April and May 2013)12. The drug was identified and authenticated at Pharmacognosy Laboratory, Institute for Post Graduate Teaching and Research in Ayurveda, Gujarat Ayurved University, Jamnagar and voucher specimen was deposited (IPGT&RA/Phm/6165). Prashasta (acceptable) and Aprashasta (unacceptable) qualities of fruits were analyzed by immersing them in a vessel containing potable water in a glass jar. The fruits that settled down were collected carefully and used for further experimental study while the floated fruits were discarded as they are unacceptable for the study.

Curing of Bhallataka

Acceptable fruits were kept in an aluminium box of 50×35.5×55 cm (L×B×H) filled with a heap of barley to maintain constant temperature and humidity for four months. Before filling barley inside the box, thermocol sheets of 10 mm width were taken and cut off with proper dimension, now these sheets were wrapped with aluminium foil of thickness of 0.009-0.03 mm and width of 250 mm and pasted to the walls of the box. Another cabin was prepared from plain thermocol sheets inside the box with 4.5 cm distanced from previously fitted aluminium foil. Yava (Barley - Hordeum vulgare) fruits were filled in the created 4.5 cm space in between and lower down the previous sheets. Now, the batches of Bhallataka were arranged in the box with alternative barley layers. Finally, barley fruits layers were arranged on the upper part and box were closed for four months. After four months, fruits were collected and processed for further use.

Bhallataka oil extraction

Both samples of Bhallataka fruits were subjected to oil extraction by Patala yantra (Pit Method)13. Bhallataka fruits were taken inside the vessel after cutting off its pseudocarp and then the mouth of the vessel was wrapped with mesh and another small vessel was fitted upon this large vessel. The whole arrangement was wrapped with mud smeared cloth and then put in a pit. Now, covered with cow-dung cakes and light was ignited. By this process, oil from fruits was extracted and collected into the small vessel.

Bhallataka Kshaudra formation process

To 20 g of Madhu (Honey), 160 g oil was added, then, 320 g Ghrita (cow ghee) was mixed and triturated for the homogeneous mixture as per Ayurvedic classic14. The same procedure was followed for the preparation of both test formulations i.e. fresh (FB) and cured (CB) samples of Bhallataka Kshaudra.

Animals

Wistar strain albino rats of either sex, weighing 200±20 g were used for the experiment. The animals were maintained under standard conditions of temperature (22±02 °C), humidity (50-60 %) and exposed to 12 hour light and dark cycles. All animals were exposed to the same environmental conditions and were maintained on a standard diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethical Committee (M.D./IAEC/14/2013/18) as per guidelines of CPCSEA, India.

Dose

The human dose of Bhallataka Kshaudra is considered as 500 mg/day15. Rat dose was calculated on basis of body surface area ratio by referring to the table of Paget and Barnes (1964)16. Based on that, the rat dose was fixed at 45 mg/kg. The test drug formulations were administered through the oral route by licking.

Experimental design

Protocol designed in the earlier study was followed in the present study with modifications as per experimental need17. Wistar albino rats of either sex were divided into five groups of six animals each. Group (I) was kept as normal control (NC) which received distilled water (10 mL/kg, po). Group (II) of animals kept as cholesterol control group, received distilled water (10 mL/kg, po) and hyperlipidaemic diet. Group (III) of animals were treated with atorvastatin (7.2 mg/kg, po) along with
hyperlipidaemic diet (SC). Group (IV) and (V) were kept as drug-treated groups which received fresh Bhallataka Kshaudra (FB) (45 mg/kg) and cured Bhallataka Kshaudra (CB) (45 mg/kg) orally along with hyperlipidemic diet.

Test formulations and vehicle were administered to respective groups in the morning hours daily for 21 days. The hyperlipidemic diet was concomitantly administered to Group (II) to (V) to induce hyperlipidemia. The hyperlipidemic diet included cholesterol suspension in coconut oil and hydrogenated vegetable oil. Cholesterol extra pure powder (SRL, Mumbai) made into 20 % suspension in coconut oil and administered orally (5 mL/kg) in the morning hours after 1 hour of drug administration while, hydrogenated vegetable oil (5 mL/kg) (Adani Wilmar Ltd., Gujarat) was administered in evening hours.

On the 22nd day, the overnight fasted rats were weighed again and blood was collected from retro-orbital puncture under light ether anaesthesia using capillary tubes, serum was separated and used for estimation of different serum biochemical parameters. An autoanalyzer (BS 200) was used for the estimations of serum total cholesterol, triglyceride, VLDL-cholesterol, Urea, creatinine, blood sugar, total protein, albumin, globulin, SGOT, SGPT and alkaline phosphatase. The rats were sacrificed and important organs like the liver, kidney, heart and aorta were excised out. The extraneous tissues were cleaned off and weight of organs was noted down except for aorta and stored in 10 % formalin solution for histopathological study.

Results
Cholesterol control, standard drug, and cured Bhallataka groups showed almost similar values of body weight gain during the experimental period while fresh Bhallataka treated group showed a decrease in body weight in comparison to initial values (Table 1). Cholesterol control group showed a non-significant increase in relative weight of liver while significant decrease ($p <0.05$) in the relative weight of kidney in comparison to normal control group (Table 1). Test drugs produced a non-significant decrease in relative weight of heart, liver and kidney except for a significant decrease in relative weight of kidney in cured Bhallataka treated group.

Cholesterol-fed rats showed statistically significant increase in total cholesterol ($p <0.05$), triglyceride ($p <0.001$), VLDL-cholesterol ($p <0.05$) and blood urea ($p <0.001$) level in comparison to normal control rats. Standard drug and both the fresh and cured Bhallataka Kshaudra showed a statistically significant decrease in cholesterol ($p <0.05$), triglyceride ($p <0.001$) and VLDL-cholesterol ($p <0.001$) level in comparison to cholesterol control group. Cured Bhallataka Kshaudra produced better effects on lipid profile and blood urea followed by fresh Bhallataka Kshaudra (Table 2).

Cured Bhallataka Kshaudra showed a statistically significant decrease while fresh Bhallataka Kshaudra produced a non-significant decrease in blood glucose level as compared to cholesterol control group whereas both the test drugs showed a significant decrease in total protein, albumin and creatinine ($p <0.05$) level in comparison to cholesterol control group (Table 2). Cholesterol-fed rats showed a significant increase in SGOT, alkaline phosphatase ($p <0.001$) and bilirubin ($p <0.05$) level in comparison to the normal control group. Both the test drugs and atorvastatin-treated groups showed a statistically significant decrease in SGOT level, total and direct bilirubin ($p <0.05$) level in comparison to cholesterol control group (Table 2).

**Table 1 — Effect of test drugs on body weight and relative organ weight of albino rats during anti-hyperlipidaemic study**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Body weight</th>
<th>Liver</th>
<th>Heart</th>
<th>Kidney</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>Relative weight (g/100 g body weight)</td>
<td></td>
</tr>
<tr>
<td>NC</td>
<td>177.33±5.90</td>
<td>188.00±4.68</td>
<td>3.377±0.186</td>
<td>0.316±0.017</td>
</tr>
<tr>
<td>CC</td>
<td>212.00±5.42</td>
<td>222.00±12.22</td>
<td>3.583±0.0804</td>
<td>0.317±0.010</td>
</tr>
<tr>
<td>SC</td>
<td>179.67±1.75</td>
<td>189.33±3.17</td>
<td>3.492±0.207</td>
<td>0.328±0.005</td>
</tr>
<tr>
<td>FB</td>
<td>214.67±7.60</td>
<td>208.83±5.20</td>
<td>3.466±0.103</td>
<td>0.298±0.007</td>
</tr>
<tr>
<td>CB</td>
<td>206.67±12.56</td>
<td>224.00±12.04</td>
<td>3.481±0.130</td>
<td>0.310±0.010</td>
</tr>
</tbody>
</table>

Data: Mean±SEM. *$p <0.05$ when compared to normal control group; ** $p <0.001$ when compared to cholesterol control group, NC—Normal Control, CC—Cholesterol control, SC—Standard control, FB—Fresh Bhallataka Kshaudra, CB—Cured Bhallataka Kshaudra.
bilirubin levels while a non-significant decrease in alkaline phosphatase level in comparison to cholesterol control group (Table 3).

Histopathological studies have shown that, control group exhibits normal cytoarchitecture in aorta, heart, kidney and liver (Fig. 1a, 2a, 3a and 4a) respectively. Cholesterol administration in rats produced mild increase in thickness of adventitia of aorta (Fig. 1b, c), moderate fatty degenerative changes in heart (Fig. 2b, c), fatty degeneration, edematous changes and intense cell infiltration in kidney (Fig. 3b, c) and liver (Fig. 4b, c). Sections from fresh and cured Bhallataka Kshaudra treated groups showed normal cytoarchitecture of aorta (Fig. 1d, e), mild fatty changes in heart (Fig. 2d, e), mild fatty changes with edema in kidney (Fig. 3d, e) and mild fatty changes in liver (Fig. 4d, e) in comparison to cholesterol control group. Standard drug treated rats showed normal cytoarchitecture of aorta (Fig. 1f), moderate fatty degenerative changes and necrosis in heart (Fig. 2f), normal cytoarchitecture of kidney (Fig. 3f) and mild fatty changes in liver (Fig. 4f).

Discussion

Bhallataka is mentioned as Medonashaka in Ayurvedic text. The previous study also suggests the anti-hyperlipidaemic effect of an extract of Bhallataka but, the difference in the fresh and cured fruits of Bhallataka prepared as per the classical method is not yet reported. The present study is carried out to evaluate the comparative anti-hyperlipidaemic efficacy of fresh and cured Bhallataka fruits in albino rats. Administration of hyperlipidaemia diet led to a marked increase in body weight, CB showed an increase in body weight but on the other hand, FB showed a decrease in body weight compared to initial values but the effect was non-significant. Cholesterol control group showed a non-significant increase in relative weight of liver and heart in comparison to control group whereas, drug-treated groups showed a non-significant decrease in relative weight of liver and heart in comparison to cholesterol control group. The weight of kidney significantly decreased in the cholesterol control group in comparison to control group. CB treated group showed significant decrease whereas, non-significant decrease in rest of the groups in comparison to cholesterol control group.

Administration of cholesterol and hyperlipidemic diet lead to significant elevation of serum total cholesterol, triglycerides, and VLDL-cholesterol

### Table 2 — Effect of test drugs on lipid profile, urea content, blood sugar, total protein, albumin and globulin level in albino rats during anti-hyperlipidaemic study

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Groups</th>
<th>NC</th>
<th>CC</th>
<th>SC</th>
<th>FB</th>
<th>CB</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol (mg/ dL)</td>
<td>46.00±3.22</td>
<td>77.33±6.36</td>
<td>56.50±2.63*</td>
<td>61.33±2.93*</td>
<td>60.33±3.461*</td>
<td></td>
</tr>
<tr>
<td>Triglyceride (mg/ dL)</td>
<td>96.50±14.05</td>
<td>176.00±9.59**</td>
<td>104.67±11.82**</td>
<td>94.00±5.42**</td>
<td>70.00±10.16**</td>
<td></td>
</tr>
<tr>
<td>VLDL (mg/ dL)</td>
<td>19.30±2.81</td>
<td>35.20±1.92**</td>
<td>20.93±2.36**</td>
<td>18.80±1.08**</td>
<td>15.20±1.97**</td>
<td></td>
</tr>
<tr>
<td>Urea (mg/dL)</td>
<td>43.50±1.48</td>
<td>61.50±1.65**</td>
<td>41.00±3.20**</td>
<td>41.67±1.26**</td>
<td>34.17±1.17**</td>
<td></td>
</tr>
<tr>
<td>Total protein (mg/ dL)</td>
<td>7.28±0.16</td>
<td>7.75±0.145</td>
<td>7.62±0.162</td>
<td>6.80±0.115**</td>
<td>6.85±0.118**</td>
<td></td>
</tr>
<tr>
<td>Albumin (mg/ dL)</td>
<td>3.88±0.083</td>
<td>3.75±0.062</td>
<td>4.05±0.085*</td>
<td>3.50±0.089*</td>
<td>3.32±0.087*</td>
<td></td>
</tr>
<tr>
<td>Globulin (mg/ dL)</td>
<td>3.40±0.13</td>
<td>4.05±0.178**</td>
<td>3.60±0.255</td>
<td>3.30±0.0775*</td>
<td>3.53±0.193</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/ dL)</td>
<td>0.55±0.02</td>
<td>0.55±0.02</td>
<td>0.53±0.03</td>
<td>0.42±0.05*</td>
<td>0.43±0.04*</td>
<td></td>
</tr>
<tr>
<td>Sugar (mg/dL)</td>
<td>103.83±5.78</td>
<td>96.00±8.32</td>
<td>96.67±5.03</td>
<td>76.17±4.06</td>
<td>72.83±2.96</td>
<td></td>
</tr>
</tbody>
</table>

Data: Mean±SEM, *p < 0.05, **p < 0.001 when compared to normal control group; #p < 0.05, ##p < 0.001 when compared to cholesterol control group

### Table 3 — Effect of test drugs on SGOT, SGPT alkaline phosphatase and creatinine level in albino rats during anti-hyperlipidaemic study

<table>
<thead>
<tr>
<th>Groups</th>
<th>SGPT (IU/L)</th>
<th>SGOT (IU/L)</th>
<th>ALP (IU/L)</th>
<th>Bilirubin (T) (mg/dL)</th>
<th>Bilirubin (D) (mg/ dL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>53.00±2.86</td>
<td>145.17±6.48</td>
<td>269.00±46.83</td>
<td>0.65±0.10</td>
<td>0.17±0.02</td>
</tr>
<tr>
<td>CC</td>
<td>52.33±3.48</td>
<td>219.83±15.76</td>
<td>535.00±87.81</td>
<td>1.07±0.08**</td>
<td>0.28±0.05**</td>
</tr>
<tr>
<td>SC</td>
<td>57.60±5.66</td>
<td>154.83±8.77*</td>
<td>426.00±46.81</td>
<td>0.62±0.05**</td>
<td>0.15±0.02*</td>
</tr>
<tr>
<td>FB</td>
<td>55.17±4.77</td>
<td>147.67±11.58*</td>
<td>409.83±59.34</td>
<td>0.48±0.03**</td>
<td>0.13±0.02*</td>
</tr>
<tr>
<td>CB</td>
<td>52.17±7.28</td>
<td>151.83±7.98*</td>
<td>494.17±79.35</td>
<td>0.52±0.06**</td>
<td>0.15±0.03*</td>
</tr>
</tbody>
</table>

Data: Mean±SEM, *p < 0.05, **p < 0.001 when compared to normal control group; *p < 0.05, **p < 0.001 when compared to cholesterol control group
normal control rats. This establishes the efficacy of the experimental protocol to induce hyperlipidemic condition. Lipid metabolism to great extent depends upon the formation and turnover of lipoproteins. Almost all lipids in the plasma are transported in the form of complexes with proteins; these proteins are termed as lipoproteins. Both test drugs significantly lowered the cholesterol level, triglyceride and VLDL cholesterol level in rats. The reversal effect was more pronounced in CB treated animals than FB treated animals in comparison to control group. The cholesterol-lowering effect of the drugs might be due to inhibition of dietary cholesterol absorption or esterification. Since two enzymes are involved in these two processes viz. pancreatic cholesterol esterase and intestinal acyl Co-A-cholesterol acyltransferase enzyme (ACAT), thus it could be suggested that the test drugs inhibit the activity of one or both of these enzymes. In both the test drugs ω-7 fatty acids were found which are responsible for lowering cholesterol level. The decrease in cholesterol may indicate increased oxidation of mobilized fatty acids of inhibition or lipolysis. CB, FB and atorvastatin produced a highly significant decrease in the triglyceride levels and VLDL level compared to the cholesterol-fed group. Reduction in cholesterol, triglycerides and VLDL level may probably be due to the presence of steroids, flavonoids and polyphenolic compounds in Bhallataka. Flavonoids are a group of ubiquitously distributed plant polyphenols which exhibit a wide range of pharmacological effects. The inhibition of lipid peroxidation is due to the free radical scavenging property of flavonoids. They scavenge free radical by donating their hydrogen groups and prevent the initiation of a chain reaction. They also scavenge singlet O₂, terminating peroxides by their low redox potential. They also reduce lipid peroxidation by reducing the levels of malondialdehyde and conjugated dienes. Both the drugs have the presence of steroids, tannins, phenolic compounds, flavonoids and amines. The plant steroids reduce the absorption of cholesterol and thus increase faecal excretion of cholesterol.

SGOT is a mitochondrial enzyme released from heart, liver, skeletal muscle and kidney. SGPT is a cytosolic enzyme primarily present in the liver. Serum alkaline phosphatase is produced by many tissues, especially bone, liver, intestine and placenta and excreted in bile. Serum amino-transferase and alkaline phosphatase are elevated in most liver
disorders. Highest elevations are found in conditions causing extensive hepatic necrosis such as severe acute viral hepatitis, toxic hepatitis or prolonged circulatory collapse. Cholesterol-fed rats exhibited an insignificant increase in SGOT and a significant increase in alkaline phosphatase ($p < 0.001$) level suggests the liver, heart and kidney damage in rats. Test drug non-significantly decreased the level of alkaline phosphatase while a significant decrease in SGOT which suggests the protective role of the test drug in high fat diet-induced hyperlipidemia in rats.

A previous study showed that increased levels of total cholesterol, free cholesterol, phospholipids, triglycerides and free fatty acids and decreased levels of ester cholesterol in plasma, liver and kidney found in cancer-suffering animals were reverted back to near normal levels on treatment with the formulation of *Bhallataka*. Further, it reduces the tissue and serum hyperlipidemia by the inhibition of intestinal cholesterol absorption coupled with peripheral disposal thus possessing anti-atherosclerotic activity. *S. anacardium* nut. extract oil fraction at a dose of 1 mg/100 g body weight significantly reduced serum cholesterol levels and increased HDL cholesterol levels in the rat fed with atherogenic diet. In this context, a number of other plants have also been reported to have antihyperglycemic, antihyperlipidemic and insulin stimulatory effects.

**Conclusion**

From the present study, it is concluded that fresh and cured *Bhallataka Kshaudra* prepared as per classical method have anti-hyperlipidaemic activity in cholesterol-fed albino rats. Cured *Bhallataka Kshaudra* has a more pronounced effect than fresh *Bhallataka Kshaudra* in lowering cholesterol and related parameters which may due to the presence of more amount of $\omega$-7 fatty acids, steroids, flavonoids and polyphenolic compounds in cured one as compared to fresh one.

**References**


