Chapter 7

Pharmacological Evaluation of *Ativiṣā* and *Mustā*

*This chapter is about the pharmacological studies conducted to evaluate the level of similarity between the AD, Ativiṣā and APD, Mustā with respect to their 3 prominent karmas*
Pharmacological evaluation of Ativiṣā and Mustā

7.1 Introduction

Mustā (C. rotundus) is mentioned as an APD for the rare herb (AD) Ativiṣā (A. heterophyllum) (Misra, 2002). This chapter explores the pharmacological similarity of this AD-APD pair. Ativiṣā is a drug used for jwara (fevers), atīsāra (diarrhoea) and medoroga (hyperlipidemia). Hence anti-hyperlipedemic and anti-diarrheal, anti-pyretic activities of oral doses of Ativiṣā and Mustā were studied using Wistar albino rat models to find the legitimacy of substitution from the pharmacological perspective.

7.2 Overall Methods

The selected dosage form of both drugs, chūrṇa (powder) is practiced in Ayurveda and traditional systems of medicine (AFI, 2003). However, neither safety, nor efficacy of this dosage form has been scientifically studied. Therefore it was planned to test the acute toxicity as per OECD guidelines (OECD 425, 2001) after taking Institutional Animal Ethical Committee (IAEC) permission.

7.2.1 Institutional Animal Ethical Committee (IAEC) Permission:

The intended study protocol was presented to the IAEC of Acharya & B.M. Reddy College of Pharmacy, Soladevanahalli, Bangalore (Reg.no. 997/c/06/CPCSEA) and obtained the approval for the same (Ref. No. IAEC/ABMRCP/2013-2014/17) (Annexure 5).

7.2.2 Animals

Wistar albino rats of either sex weighing between 150-180 g were procured from the animal house of Acharya & BM Reddy College of Pharmacy, Bangalore. They were housed in polyacrylic cages containing rice husk under standard environmental conditions (27±2°C and
relative humidity 44-56 \%). Animals were fed standard diet and had free access to water (Kulkarni, 2009).

7.2.3 Test drugs

The test drugs, namely tubers of *Aconitum heterophyllum* and rhizomes of *Cyperus rotundus* were collected and authenticated by the qualified field botanists and voucher specimens deposited in FRLHT herbarium. Cleaned samples were dried in a dehydrator at 40°C under shade and finely powdered (IS No. 80 mesh) using electrical pulverizer. Based on the human doses of the study drugs as indicated in the Ayurvedic Pharmacopoeia of India (API) (API, 2001a; API 2000b), doses for rats were derived using the standard animal dose conversion formula.

\[
\text{Animal dose (mg/kg)} = \text{Human dose (mg/kg)} \times \text{Conversion factor.}
\]

The conversion factor for rats (~ 150 g) is 6.77 (Shin et al., 2010). Fine powders of the test drugs were suspended (80mg/ml) in 0.1 % CMC solution and orally fed to the animals.

7.2.4 Acute oral toxicity studies

The purpose of conducting acute oral toxicity studies is to determine the nature and extent of the untoward reactions which might occur, following the administration of a single or overdose of the drug.

Acute oral toxicity study was performed as per the guidelines of Organization for Economic Cooperation and Development (OECD- 425, 2001). After oral administration of the test drug, animals were observed individually for behavioral profile (alertness, restlessness, irritability and fearfulness), neurological profile (spontaneous activity, reactivity, touch response and
pain response) and autonomic profile (defecation and urination) for at least once during the first 30 min and periodically during the first 24 h, with special attention given during the first 4 h and daily thereafter, for a total of 14 days. A total of six Swiss albino mice (either sex) were used and each received a single oral dose of 2000 mg/kg/p.o. (limit test). Animals were kept overnight fasting prior to drug administration and food was withheld for further 3-4 h.

7.3 Experimental protocol for anti-hyperlipedemic, anti-diarrheal and anti-pyretic activity studies

Wistar Albino rats of either sex weighing between 150-180g were used in the study. The Experimental protocol is as follows:

<table>
<thead>
<tr>
<th>S. No</th>
<th>Group</th>
<th>Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Group 1</td>
<td>Served as control, received 2 ml 0.1% CMC solution orally.</td>
</tr>
<tr>
<td>2.</td>
<td>Group 2</td>
<td>Rats orally received respective standard drug doses in 0.1 % CMC, as standard treatment.</td>
</tr>
<tr>
<td>3.</td>
<td>Group 3</td>
<td>Rats orally received 200 mg/kg <em>Aconitum heterophyllum</em> powder suspended in 0.1 % CMC.</td>
</tr>
<tr>
<td>4.</td>
<td>Group 4</td>
<td>Rats orally received 400 mg/kg <em>Aconitum heterophyllum</em> powder suspended in 0.1 % CMC.</td>
</tr>
<tr>
<td>5.</td>
<td>Group 5</td>
<td>Rats orally received 400 mg/kg <em>Cyperus rotundus</em> powder suspended in 0.1 % CMC.</td>
</tr>
<tr>
<td>6.</td>
<td>Group 6</td>
<td>Rats orally received 800 mg/kg <em>Cyperus rotundus</em> powder suspended in 0.1 % CMC.</td>
</tr>
</tbody>
</table>
7.3.1 Anti-hyperlipidemic activity of *A. heterophyllum* and *C. rotundus*

Imbalanced diet and lack of physical exercise are considered to be the causative factors for obesity and hyperlipidemia (Mopuri 2010). Hyperlipidemia are the major risk factors for cardiovascular diseases, atherosclerosis, myocardial infarction, heart attacks and cerebrovascular diseases (Megalli et al., 2005). Hyperlipidemia is characterized by an abnormal elevation of the serum lipids such as total cholesterol (TC), low-density lipoprotein-cholesterol (LDL) and triglycerides (TG) (Na-Young, 2008). In recent years there have been several attempts to discover novel molecules which can prevent hyperlipidemia as well as bring down the increased serum lipids from single or polyherbal medicines Hydroxycitric acid (Megalli et al., 2005).

It is worth noting that, the study drugs, *Ativiṣā* (*A. heterophyllum*) is referred to in Ayurveda as an anti-hyperlipidemic agent (*Medohara*) (Sastry, 1997a). It is also an extensively prescribed anti-lipidemic agent. The substitute, *Mustā* (*C. rotundus*) is also found to be used as an anti-hyperlipidemic agent (Chunekar, 2004). Hence the study was undertaken to determine their anti-hyperlipidemic activity in hyperlipidemic Wistar albino rats. Hyperlipidemic rats were fed with crude drug powders of 200 mg/kg and 400 mg/kg of *A. heterophyllum* and 400 mg/kg and 800 mg/kg of *C. rotundus* respectively for 30 days. Total Cholesterol (TC), Triglycerides (TG), High density Lipoprotein (HDL), Low Density Lipoprotein (LDL), Very Low Density Lipoprotein (VLDL), Blood Sugar and Body Weight of the animals were monitored to assess the anti-hyperlipidemic potential of the test drugs.
7. 3.1.2 Methods

Method followed to study the anti-hyperlipidemic activity of test drugs is adopted from the works of Na Young Yoon (2008), Chandratre et al. (2011) and Senecha and co-workers (2012). Thirty six Wistar albino rats of either sex weighing around 180-200g were selected. A high fat diet, 'Nutrilab Rodent feed' was purchased from Provimi Nutrition India Pvt. Ltd., Bangalore (Composition: crude protein 21 %, crude fat 10%, crude fiber 5%, calcium 1 %, Phosphorus 0.7%, total ash 7%) and was used to substitute normal diet for the rodents for 6 weeks. After 6 weeks serum was obtained from retro-orbital sinus blood and by centrifuging for 10 minutes @ 3000 rpm. Induction of hyperlipidemia was confirmed by analyzing the biochemical parameters viz., Total Cholesterol (TC), Triglycerides (TG), High density Lipoprotein (HDL) of the animals. Serum analysis was done using a Semi-Automatic Biochemistry Analyser (Mispa Excel, Mumbai) and relevant lipid profile diagnostic kits (Agappe diagnostics, Mumbai) purchased in Bangalore.

Low Density Lipoprotein (LDL) and Very Low Density Lipoprotein (VLDL) cholesterol were calculated with reasonable accuracy using the Friedewald Formula

\[ \text{LDL} = \text{Total Cholesterol} - \text{HDL} - \left( \frac{\text{Triglycerides}}{5} \right) \]

\[ \text{VLDL} = \frac{\text{Triglycerides}}{5} \]

After confirming induction of hyperlipidemia, rats were randomly divided into six groups of six animals each. Treatment or test Group consisted of rats which were oral fed with the drugs for 30 days along with high fat feed. On 31st day, animals were weighed. Serum was collected from overnight fasted animals and subjected for biochemical tests. Positive control for the study was Simvastatin (5 mg/kg) (Chandratre et al., 2011; Senecha et al., 2012)
7.3.2 Anti-diarrhoeal activity of *A. heterophyllum* and *C. rotundus*

Even today, diarrhoea and dysentery are leading causes of malnutrition and deaths among children in the developing countries of the world (Victoria et al., 2000). The drugs, *A. heterophyllum* and *C. rotundus* are claimed to have ‘grāhi’ property (preventing water loss from the body). Compound formulations of *A. heterophyllum* and *C. rotundus* like *Balachaturbhadra chūma* and *Mustārishta* are very often prescribed by Ayurvedic practitioners as a remedy to diarrhoea and dysentery. (AFI, 2000; Chunekar, 2004).

Hence this study was undertaken to determine anti-diarrhoeal activity of *A. heterophyllum* and *C. rotundus* in the castor oil induced diarrhea in Wistar albino rats. Episodes of defecation, wet defecation, fecal weight after 4 hours, and post induction delay in defecation were evaluated to assess the anti-diarrhoeal potential of the test drugs.

7.3.2.1 Methods

The anti-diarrhoeal study was conducted as described by Rao and Lakshmi (2012) and Akuodor et al. (2011). Thirty six Wistar albino rats of either sex were acclimatized for 7 days with normal food, water and housing. They were divided into six groups of six animals each and fasted for 24 hours prior to the test, but allowed free access to water. Drugs were administered orally. Animals were fed with crude drug powders of 200 mg/kg and 400 mg/kg of *A. heterophyllum* and 400 mg/kg and 800 mg/kg of *C. rotundus* respectively. The animals were housed singly in cages lined with tissue paper. One hour after pre-treatment with the crude powder, the animals were challenged with 1 ml of castor oil orally. Thereafter, they were observed for 4h for the parameters like delay in onset of diarrhoea (in minutes), total episodes of defecation, episodes of diarrhoea, mass of fecal matter (g) and animal behavior. Positive control for the study was Loperamide 3mg/kg body weight.
7.3.3 Anti-pyretic activity of *A. heterophyllum* and *C. rotundus*

*Mustā* (*C. rotundus*) has been considered to be one of the choices of drugs (*Agryadravya*) in treatment of fevers (*Mustā parpatakau jware*) (Sastry, 1997a). It is also extensively prescribed by Ayurvedic practitioners in different dosage forms in managing fevers (Chunekar, 2004; AFI, 2003). *Ativiṣā* (*A. heterophyllum*) is also an ingredient in several anti-pyretic formulations. However, *Vatsanābha* (*Aconitum ferox*) of same family (Rananculaceae) is extensively prescribed anti-pyretic drug. With the scientific curiosity to investigate if *A. heterophyllum* also exhibit anti-pyretic actions similar to that of *C. rotundus*, this particular study was taken up.

7.3.3.1 Methods

The method reported by Padhan et al. (2010) and Patra et al. (2009) to evaluate anti-pyretic activities of herbal drugs was followed in this study. Thirty six Wistar albino rats of either sex were acclimatized for 7 days with normal food, water and housing. By insertion of a digital thermometer to a depth of 2 cm into the rectum the initial rectal temperatures were recorded. Those animals with normal body temperature (37 °C) only were selected for the study.

One day prior to the study, 10 mL/kg of Brewer’s yeast (Hamsa Biosciences, Bangalore) suspension (15% w/v in 0.9% saline) was injected subcutaneously below the nape of the neck. The site of injection was massaged in order to spread the suspension beneath the skin. Immediately after yeast injection, food was withdrawn. 18 hours post challenge, the rise in rectal temperature was recorded. The measurement was repeated after 30 min. Only animals with a body temperature of at least 38 °C (100.4 ° F) were taken into the study. The animals with pyrexia were divided into six groups of six animals each. All interventions
were administered orally. Rectal temperatures of the animals were recorded 60, 120, 240 and 360 minutes post treatment. During the study, animals were observed constantly for their activities and behavior. Positive control for the study was Paracetamol 150mg/kg body weight.

7.3.4 Statistical Analysis
All values were expressed as mean±SEM (n=6), the results were analyzed by Analysis of Variance (ANOVA) followed by Tukeys post hoc test, using software Prism (version 5). Results * \( P < 0.05 \) compared to the control was considered statistically significant.

7.4 Results
7.4.1 Acute oral toxicity studies
Neither mortality nor any signs of toxicity (in behavioral, neurological profile and autonomic profile; Section 7.2.4) were observed up to dose of 2000mg/kg body weight among the animals during the observation period. Therefore, the test drugs were assumed to be safe. Indicated human dose for \( A. \) heterophyllum is 2-3 g. (API, 2001a) and for \( C. \) rotundus 4-6 g. (API, 2001b). On this basis, the test doses were fixed as 200 and 400 mg/kg of \( Aconitum heterophyllum \) and 400 and 800 mg/kg of \( Cyperus rotundus \) crude drug powders for oral the administration in rats.

Since the drugs were found to be safe up to 2000 mg/kg, it was proceeded to study the pharmacological activities.
7.4.2 Anti-hyperlipidemic activity of *A. heterophyllum* and *C. rotundus*

Results of anti-hyperlipidemic activity of *A. heterophyllum* and *C. rotundus* are presented in Table 7.1 and Figures 7.1 to 7.8.

**Table 7.1:** Anti-hyperlipidemic effect of *A. heterophyllum* (A.h) and *C. rotundus* (C.r) oral administration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Cholesterol (mg/dL)</th>
<th>Triglycerides (mg/dL)</th>
<th>HDL (mg/dL)</th>
<th>LDL (mg/dL)</th>
<th>VLDL (mg/dL)</th>
<th>Blood sugar (mg/dL)</th>
<th>Body weight (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td>115.6±3.35</td>
<td>95.42±2.78</td>
<td>30.97±0.84</td>
<td>65.53±3.49</td>
<td>19.08±0.55</td>
<td>149.0 ± 4.07</td>
<td>204.0±8.86</td>
</tr>
<tr>
<td>Simvastatin (5mg/kg)</td>
<td></td>
<td>84.09±1.92* ** ***</td>
<td>71.78±2.42*</td>
<td>40.13±0.79</td>
<td>29.61±1.78* ** ***</td>
<td>14.36±0.48***</td>
<td>100.8 ± 5.33***</td>
<td>180.8±4.73</td>
</tr>
<tr>
<td>A.h (200 mg/kg)</td>
<td></td>
<td>93.77±3.01* **</td>
<td>75.66±3.26*</td>
<td>39.05±1.92*</td>
<td>39.59±2.13* **</td>
<td>15.13±0.65**</td>
<td>123.3 ± 4.74*</td>
<td>181.7±5.27</td>
</tr>
<tr>
<td>A. h (400 mg/kg)</td>
<td></td>
<td>84.35±2.84* **</td>
<td>72.64±3.14*</td>
<td>38.35±1.97*</td>
<td>31.47±2.16* **</td>
<td>14.53±0.63**</td>
<td>94.50 ± 3.79***</td>
<td>172.5±3.82**</td>
</tr>
<tr>
<td>C.r (400 mg/kg)</td>
<td></td>
<td>88.36±3.53*</td>
<td>74.93±3.62*</td>
<td>36.17±1.26*</td>
<td>37.46±2.08* **</td>
<td>14.99±0.73**</td>
<td>98.00 ± 6.48**</td>
<td>186.7±5.43</td>
</tr>
<tr>
<td>C.r (800 mg/kg)</td>
<td></td>
<td>90.03±2.30*</td>
<td>78.46±3.64*</td>
<td>39.46±0.69*</td>
<td>34.88±2.15* **</td>
<td>15.69±0.73**</td>
<td>92.17 ± 5.36***</td>
<td>187.5±5.59</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; n=6; * P < 0.05, ** P < 0.01, ***P < 0.001 w.r.t control.
**Figure 7.1:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on total Cholesterol (TC)

**Figure 7.2:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on Triglycerides (TG)
**Figure 7.3:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on High Density Lipoprotein (HDL)

**Figure 7.4:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on Low Density Lipoprotein (LDL)
Pharmacological evaluation of *Ativiṣā* and *Mustā*

**Figure 7.5:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on Very Low Density Lipoprotein (VLDL)

**Figure 7.6:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on blood sugar
Pharmacological evaluation of *Ativiṣā* and *Mustā*

**Figure 7.7:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on body weight

**Figure 7.8:** Comparison of effect of crude powders of *A. heterophyllum* and *C. rotundus* on HDL and LDL Cholesterol
In the present study, the model selected was mimicking the real life scenario. Animals were brought to hyperlipidemia gradually and naturally, by high fat diet. By 45 days of high fat diet, rats achieved hyperlipidemia and hyperglycemia. During the treatment fat rich diet was continued. Both *A. heterophyllum* at 400 mg/kg and *C. rotundus* 800 mg/kg dose showed significant (*P*<0.05) anti-hyperlipidemic activity compared to the control.

### 7.4.2.1 Discussion

In the present investigation, a significant anti-hyperlipidemic activity of crude drug powders of both *A. heterophyllum* and *C. rotundus* was observed, in terms of multiple lipid profile markers. Being the major cause of life-threatening coronary heart disease, hyperlipidemia is one of the conditions of major focus in present day drug discovery (Senecha et al., 2012). This study has shown that, the crude powders of *A. heterophyllum* and its APD, *C. rotundus*, are capable of decreasing diet induced hyperlipidemia. Compared to the control (115.6±7.49 mg/dL), *A. heterophyllum* at doses of 200mg/kg and 400 mg/kg lowered total cholesterol (93.77±7.39 mg/dL, *P* < 0.01; 84.35 mg/dL; *P* < 0.001 respectively). *C. rotundus* also controlled cholesterol (88.36±7.91 mg/dL and 90.03±5.63 mg/dL) significantly (*P* < 0.01). However, compared to *A. heterophyllum*, *C. rotundus* was less active.

All 4 test groups showed significantly lower triglycerides compared to control (95.42±6.20 mg/dL). However, the activity of *A. heterophyllum* (72.64±7.69 mg/dL; *P* < 0.001) was better compared to *C. rotundus* (74.93±6.12 mg/dL; *P* < 0.01). As additional tests, Fasting blood sugar and body weight were also compared. Both groups of *C. rotundus* showed significant reduction (98.00 ± 14.49 mg/dL and 92.17 ± 13.15 mg/dL; both *P* < 0.01) in FBS compared to control (149.0 ± 9.11 mg/dL), whereas only *A. heterophyllum* 400 mg/kg reduced blood sugar (94.50 ± 9.29 mg/dL; *P* < 0.001) significantly.
Different extracts of both drugs have been reported to have anti-hyperlipidemic and anti-obesity activities (Lemaure et al., 2007, Chandratre et al., 2011; Chandratre et al., 2012). However, the crude drugs (the form, which used in Ayurveda and other traditional Indian systems of medicine) have not been studied earlier. The methanolic extract of A. heterophyllum had lowered total cholesterol, triglycerides and apolipoprotein B in diet induced obese rats, but increased HDL-cholesterol and apolipoprotein A. (Subash and Augustine, 2012). Hexane extracts of C. rotundus tubers reduced weight gain by 10% in diet induced obese Zucker rats. It was suggested that the effect was due to activation of the β3-AR (adrenoreceptor) by the extract (Lemaure et al., 2007).

The aqueous and alcoholic extracts of the rhizomes of C. rotundus significantly lowered total serum cholesterol, triglyceride and low density lipoprotein levels. However, the specific constituents responsible for the activity were not reported (Chandratre et al., 2011; Chandratre et al., 2012). Anti-hyperglycemic effect of the hydro-alcoholic extract of Cyperus rotundus was also reported in alloxan induced hyperglycemic rats (Raut et al., 2006). The methanolic extract of C. rotundus inhibited the enzymes alpha-glucosidase and alpha-amylase, involved in carbohydrate digestion. A new flavane, (2RS,3SR)-3,4',5,6,7,8-hexahydroxyflavane and stilbene dimers cassigerol E and scirpusins A and B were identified as the compounds responsible for the activity (Thi-Tran et al., 2014).

Both drugs have the dominance of tikta rasa (bitter taste), laghu (light to digest) and rūkṣa (dry) properties, which directly helps to reduce kapha component and medo dhātu (adipose tissue) from the body. A therapeutically useful medohara dravya (anti-lipidemic drug), need to decrease excessive medo dhātu from the body, yet, not causing emaciation or wasting of
any other tissue, especially *mamasa dhātu* (muscle tissue) (Fig. 5.4; section 5.3.1.3). *Mustā* (*C. rotundus*) is a *lekhanīya* and *medohara dravyas* commonly used by Ayurvedic physicians (Sastry 1997). *Ativiṣā* may be a much more efficient anti-lipidemic agent, but may deplete muscle tissue and *rasa dhātu* (chyle/plasma), on chronic use. Therefore, it is much safer to use *C. rotundus*.

### 7.4.2.1 Conclusion

Crude drug powders of both *A. heterophyllum* and *C. rotundus* are capable of controlling high fat diet induced hyperlipidemia, hyperglycemia and weight gain in rats. They lowered total cholesterol, triglycerides, LDL and VLDL, blood sugar and body weight compared to the control. Since the anti-hyperlipidemic activity of *A. heterophyllum* and *C. rotundus* are not different on statistical comparison, probably, the more easily available *C. rotundus* could substitute rare and endangered *A. heterophyllum* to manage obesity and lipidemia. However, further studies are required to examine the method of action, dosage optimization and pathways involved in this activity.
7.4.3 Anti-diarrhoeal activity of *A. heterophyllum* and *C. rotundus*

The results of the anti-diarrhoeal activity of *A. heterophyllum* and *C. rotundus* are presented in Table 7.2 and figures 7.9 to 7.12 respectively.

**Table 7.2:** Anti-diarrhoeal activity of *A. heterophyllum* (A.h) and *C. rotundus* (C.r) oral administration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Parameters</th>
<th>Episodes of defecation in 4 h (no.)</th>
<th>Episodes of wet defecation (no.)</th>
<th>Fecal weight (g)</th>
<th>Delay in defecation (min.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Challenge</td>
<td></td>
<td>11.33 ± 0.76</td>
<td>8.67 ± 0.88</td>
<td>5.20 ± 0.31</td>
<td>84.67 ± 6.46</td>
</tr>
<tr>
<td>Loperamide (3mg/kg)</td>
<td></td>
<td>2.40 ± 0.40***</td>
<td>1.33 ± 0.33***</td>
<td>1.70 ± 0.28 ***</td>
<td>177.5 ± 8.55 ***</td>
</tr>
<tr>
<td>A. h (200 mg/kg)</td>
<td></td>
<td>8.0 ± 0.82</td>
<td>4.83 ± 0.60**</td>
<td>3.74 ± 0.10</td>
<td>123.8 ± 5.74 **</td>
</tr>
<tr>
<td>A. h (400 mg/kg)</td>
<td></td>
<td>7.16 ± 0.79</td>
<td>4.95 ± 0.48*</td>
<td>3.48 ± 0.46</td>
<td>128.3 ± 8.11 **</td>
</tr>
<tr>
<td>C. r (400 mg/kg)</td>
<td></td>
<td>9.50 ± 1.20</td>
<td>5.83 ± 0.87</td>
<td>3.31 ± 0.38</td>
<td>93.33 ± 6.14</td>
</tr>
<tr>
<td>C. r (800 mg/kg)</td>
<td></td>
<td>8.00 ± 0.86</td>
<td>5.0 ± 0.63*</td>
<td>2.59 ± 0.47*</td>
<td>105.3 ± 7.59</td>
</tr>
</tbody>
</table>

Values are expressed as mean±SEM; n=6; * P < 0.05, ** P < 0.01, ***P < 0.001 w.r.t control.
**Figure 7.9**: Effect of crude powders of *A. heterophyllum* and *C. rotundus* on episodes of defecation in 4 h

**Figure 7.10**: Effect of crude powders of *A. heterophyllum* and *C. rotundus* on episodes of wet defecation
Pharmacological evaluation of *Ativiṣā* and *Mustā*

**Figure 7.11:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on total weight of fecal matter in 4 h

**Figure 7.12:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on onset of diarrhea
In the present study, both *A. heterophyllum* and *C. rotundus* were showed to have anti-diarrhoeal action at doses of 400 mg/kg and 800 mg/kg respectively, as there was significant reduction in diarrhoeal episodes compared to the control (*P* < 0.05). Though, *A. heterophyllum* appeared to be better anti-diarrhoeal drug, compared to *C. rotundus*, the difference between them was not statistically significant.

### 7.4.3.1 Discussion

During the present study, *A. heterophyllum* at both 200mg/kg and 400 mg/kg doses effectively controlled diarrhea by delaying onset of diarrhea (123.8± 5.74 and 128.3± 8.11 min) compared to control (84.67± 6.46 min) (*P* < 0.05). Both doses of *C. rotundus* did not show a significant delay in onset of diarrhoea. Both *A. heterophyllum* 400 mg/kg and *C. rotundus* 800 mg/kg controlled episodes of diarrhea (4.95± 0.48 and 5.0± 0.63 nos. respectively), compared to the control group animals (8.67 ± 0.88 nos.) (*P* < 0.05).

Alcoholic extracts of both *A. heterophyllum* and *C. rotundus* were proved to have anti-diarrhoeal activity in mice models (Venkatasubramanian et al, 2010). The activity of *A. heterophyllum* was higher compared to that of *C. rotundus*. Aqueous extract of *C. rotundus* effectively inhibited labile toxin and stable toxin production from *E. coli*, EPEC (enteropathogenic) and ETEC (enterotoxigenic) (Daswani et al., 2001). Limited anti-bacterial / anti-rotaviral activity of *C. rotundus* was reported and its anti-diarrhoeal effect was related to its action on some feature of bacterial virulence such as colonization, production of cholera toxin or labile toxin (Birdi et al., 2011). The methanol extract was also reported to suppress the frequency of the diarrhoeal episodes and prolong the latent period for diarrhoeal onset in mice (Uddin et al., 2006).
Grāhi karma (preventing water-loss from the body) is responsible for stopping diarrhoea. Ativiṣā is uṣṇa and grāhi (hot in potency and prevents water los from body), dipana and pāchana (digestive). Mustā is stambhaka (checks water loss from the body because of constriction action of coldness) (Fig. 5.2; section 5.3.1.3). Overall, Ativiṣā may be of much use in diarrhoea with indigestion, whereas Mustā may be used in diarrhoea caused by inflammatory changes in intestine.

7.4.3.2 Conclusion

Both A. heterophyllum and C. rotundus powders controlled diarrhoea in rats. They delayed onset of diarrhea and reduced watery diarrhoeal episodes, compared to the control \( (P < 0.05) \). Their activities are not statistically differing each other. Therefore, it may be worth to substitute C. rotundus for A. heterophyllum to manage diarrhoeal disorders. The mechanism of action and related chemical constituents in both drugs needs to be explored.
7.4.4 Anti-pyretic activity of *A. heterophyllum* and *C. rotundus*

The initial body temperature (°F), pyrexia after induction of fever, temperature variation in different groups of animals treated with different doses of *A. heterophyllum* and *C. rotundus* are presented in Table 7.3 and Figures 7.13 to 7.18 respectively. Figure 7.19 compares the antipyretic activities of *A. heterophyllum* and *C. rotundus* at different time intervals.

**Table 7.3:** Anti-pyretic activity of *A. heterophyllum* (A.h) and *C. rotundus* (C.r) oral administration in rats

<table>
<thead>
<tr>
<th>Groups</th>
<th>Normal temperature (prior to induction) °F</th>
<th>Temperature in °F 18 h + after induction</th>
<th>Temperature after treatment in °F</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>0 h</td>
<td>1st hr</td>
</tr>
<tr>
<td>Control</td>
<td>98.97± 0.25</td>
<td>101.6± 0.43</td>
<td>101.6± 0.48</td>
</tr>
<tr>
<td>Paracetamol 150mg/kg</td>
<td>98.88± 0.14</td>
<td>101.4± 0.29</td>
<td>99.70± 0.35**</td>
</tr>
<tr>
<td>A.h (200 mg/kg)</td>
<td>98.63± 0.24</td>
<td>102.0± 0.30</td>
<td>101.0± 0.15</td>
</tr>
<tr>
<td>A. h (400 mg/kg)</td>
<td>98.85± 0.18</td>
<td>101.7± 0.35</td>
<td>100.5± 0.15</td>
</tr>
<tr>
<td>C.r (400 mg/kg)</td>
<td>98.8± 0.25</td>
<td>101.7± 0.32</td>
<td>100.9± 0.20</td>
</tr>
<tr>
<td>C.r (800 mg/kg)</td>
<td>98.67± 0.19</td>
<td>101.8± 0.29</td>
<td>100.4± 0.22 *</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SEM; n=6; * P < 0.05, ** P < 0.01, ***P < 0.001 w.r.t control.
Pharmacological evaluation of Ativiṣā and Mustā

**Figure 7.13**: Initial (before induction) body temperature of animals

**Figure 7.14**: Body temperature of animals at '0' hour of the study
**Figure 7.15:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on pyrexia at the end of 1\textsuperscript{st} hour

**Figure 7.16:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on pyrexia at the end of 2\textsuperscript{nd} hour
**Figure 7.17:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on pyrexia at the end of 4th hour

**Figure 7.18:** Effect of crude powders of *A. heterophyllum* and *C. rotundus* on pyrexia at the end of 6th hour
In the present study, all 36 animals got pyrexia (100.4 °F) or more by 18 hours after injection of yeast suspension. Both study drugs had milder anti-pyretic activity and slow to act compared to Paracetamol. By the end of 2nd hour, both *A. heterophyllum* at 400 mg/kg and *C. rotundus* at 800 mg/kg doses controlled fever (100.3±0.7 °F; *P* < 0.05 and 99.95±0.9 °F; *P* < 0.01 respectively). The temperature difference between control (101.6±1.0 °F) and test drugs were statistically significant and *C. rotundus* was efficient compared to *A. heterophyllum* at that dose. During the study period, the animals treated with *C. rotundus* were active compared to those animals received *A. heterophyllum*. 

**Figure 7.19:** Comparison of anti-pyretic activities of *A. heterophyllum* and *C. rotundus* in different time intervals
7.4.4.1 Discussion

Crude drug powders of *A. heterophyllum* (400 mg/kg) and *C. rotundus* (800 mg/kg) were effectively controlled fever, though the onset of action was slow compared to the standard drug. Different extracts of *A. heterophyllum* have been reported to have anti-pyretic and anti-inflammatory activities, though not comparable with modern anti-pyretics. (Ikrum et al., 1987). Its alkaloids are considered to be the active molecules responsible for anti-inflammatory activity (Verma et al., 2010). Studies established the anti-inflammatory activity of different extracts of *C. rotundus* (Biradar et al., 2010, Tsoyi et al., 2011). Cyperone, one of the sesquiterpene present in *C. rotundus*, was showed to be the responsible factor to inhibit COX-2 expression and PGE-2 production (Jung S–H 2013). All these reports support the traditional claim of *Cyperus rotundus* as an anti-pyretic agent. Compound formulations like *Mustākarishata* and *Shadangapaneeeya* contain *Mustā* and used Ayurveda to treat fevers. *Ativiśā* is also an ingredient in several anti-pyretic formulations like Balachaturbhadrika chūrṇa, Sudarshana Chūrṇa and Lakshminarayana Rasa (AFI, 2000).

Both drugs are capable of amapachana (clearing *ama ≈ toxins*) and *srotoshodhana* (clearing channels/vessels) property. Formation of *ama* and blockage of *srotas* (channels) is considered to be the major factors in fever pathophysiology. *Kaṭu rasa* (pungent taste) of both drugs causes *swedajanana* (sweating), which relieves pyrexia (Figure 5.3; section 5.3.1.3).
7.4.4.2 Conclusion

As indicated in Ayurvedic texts and practiced contemporarily, *C. rotundus* has been shown to be a drug of choice to control fever. A similar action of *A. heterophyllum*, demonstrates that, probably Ayurvedic indication of AD-APD are mutually exchangeable i.e., *C. rotundus* can replace *A. heterophyllum* and vice versa.

7.5 Overall conclusion

On nut shell, both *Ativiṣā* (*A. heterophyllum*) and *Mustā* (*C. rotundus*) have shown significant anti-hyperlimidemic, anti-diarrhoeal and anti-pyretic actions when compared to the controls. Ayurveda’s approach towards drugs is based on pharmacodynamics rather than morphology or chemical constituents. The above discussed pharmacological studies supports the possibility of substituting *Mustā* (*C. rotundus*) instead of *Ativiṣā* (*A. heterophyllum*). Extensive studies with multiple pharmacological study models and clinical trials may further concretise the concept.