Total phenolic and flavonoid content, antioxidant effects and antidiarrheal activity of balacaturbhadrika churna – an Ayurvedic preparation

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Received 15 January 2019; revised 30 April 2019

Balacaturbhadrika churna (BC) is an important pediatric preparation in Ayurveda. It is used to treat various diseases of gastro-intestinal tract particularly diarrhea in children. Free radicals cause oxidative damage in the intestine and are correlated with its diseases. In the current study, total phenolic and flavonoid contents of BC extracts were determined and their antioxidant evaluation in various in vitro models was carried out. Procedures mentioned in The Ayurvedic Formulary of India were followed to prepare in-house BC; alcoholic (BCAL) and aqueous (BCAQ) extracts were prepared and tested by standard procedures. BCAL was tested for antidiarrheal activity in castor oil induced model. The ethanolic extract showed better antioxidant activity in many of the models as compared to aqueous extract. The present study showed substantial amounts of flavonoids and phenolics in churna extracts. BCAL showed significant antidiarrheal activity against the tested model. The potential antioxidant and antidiarrheal activities displayed by Balacaturbhadrika churna extracts could be attributed to these contents.

Keywords: ABTS, Antidiarrheal, Antioxidant, Balacaturbhadrika churna, DPPH, Flavonoid, Phenolic

IPC Code: Int. Cl.¹⁹: A61K 39/395, A61K 9/00, A61P 17/18, A61K 36/00, A61K 8/97, C12G 3/05, A61K 31/05

Free radicals are highly reactive oxygen species viz generation of superoxide ion, singlet oxygen, hydroxyl ion and hydrogen peroxide occur in the cells during metabolism. They cause tissue injury by producing oxidative damage to lipids, enzymes, proteins as well as DNA through covalent binding. Naturally occurring antioxidant agents have gained much attention due to their ability to scavenge free radicals¹. Antioxidant properties from Plant based phenolics as well as flavonoids have been reported regularly². Several gastrointestinal diseases have been correlated to free radical species and their damage due to oxidation in the intestine³. These free radicals directly or indirectly play a role in asthma also⁴.

Ayurveda is gaining importance globally under traditional medicines and are accepted for their clinical efficacy. BC is a reputed classical Ayurvedic pediatric preparation used in the management of gastrointestinal disorders like as diarrhea, emesis and cough. It is also used to treat asthma, fever and emaciation in children⁵. The literature survey has revealed that the various ingredients of churna have been reported to have shown either antidiarrheal activity or antibacterial activity against the diarrhea causing organisms. The first ingredient of churna viz Cyperus rotundus decoction exhibited commendable effect on diarrhea causing pathogens in in vitro models⁶. The second, third and fourth ingredients of the churna namely Pistacia integerrima galls extract, Piper longum essential oils and Aconitum heterophyllum have reported to possess antibacterial activity against diarrhea causing E. coli⁷,⁸,⁹. However, no in vivo study has been conducted on churna or its extracts directly for its antidiarrheal activity in experimental animals. It was therefore, thought worthwhile to test extract of churna for its claimed activity. Our study was thus, envisaged to estimate the total phenolic as well as flavonoid contents. The study also was to investigate the antioxidant capacity and antidiarrheal activity of the extracts of BC.
Methodology

Plant materials and chemicals
Western ghats form a major habitat for flora and the crude drugs that was obtained from the local market of Ayurvedic shop in Udupi district, Karnataka, India. The crude drugs were authenticated by the botanist Usharani K Suvarna, Department of Botany, MGM College, Udupi, Karnataka, India and the voucher specimens of each crude drug (PP575A, PP606, PP604 and PP605) has been deposited in the museum of the Department of Pharmacognosy, Manipal College of Pharmaceutical Sciences, Manipal, India. ABTS and DPPH were procured from Sigma Chemicals, USA. Analytical grade chemicals were used for experimental purpose. Castor oil was procured from Suvidhinath Laboratories, Baroda. Propylene glycol was obtained from Universal Laboratories Private Limited, Mumbai and Loperamide capsule (standard) used is from Janssen-Cilag Pharmaceuticals, Mumbai.

Churna preparation
Procedures mentioned in The Ayurvedic Formulary of India were followed to prepare in-house BC. Equal proportions of ingredients from the rhizomes of Musta (Cyperus rotundus), galls of Karkatasringi (Pistacia integerrima), fruits of Pippali (Piper longum) and root tubers of Ativisa (Aconitum heterophyllum) were powdered individually using cyclone mill and sieved through #80. The powdered ingredients were then mixed to get uniformly blended churna.

Preparation of alcoholic extract (BCAL)
Continuous hot extraction process was carried out to extract 30 g of BC using ethanol (90%). A desiccator was used to store the dried extract (28.93%) until its use.

Preparation of aqueous extract (BCAQ)
Chloroform water (2.5 mL of chloroform in 1000 mL of purified water) was used to macerate 30 g of BC. The process was carried out for seven days following which the contents were filtered and evaporated to dryness. A desiccator was used to store the dried extract (14.9%) until its use.

Determination of total phenolic content
Extract samples were taken and estimated their total phenolic content by Folin-Ciocalteau reagent method. In the process, 2.5 mL and 2 mL of Folin-Ciocalteau reagent (1/10 dilution) and 7.5% sodium carbonate (w/v) respectively were added to 0.5 mL of each extract in concentrations of 125, 250, 500, 1000 µg. The mixture was kept aside for 0.5 h with intermittent shaking during which a blue color was formed. The intensity of the color developed was measured at 765 nm using UV-Vis Spectrophotometer (UV-1650PC, Shimadzu Corp., Kyoto, Japan). A control tube with sodium carbonate (2.55 mL of distilled water+2 mL of 7.5% sodium carbonate) along with 2 mL of Folin-Ciocalteau reagent was used as blank. A standard Gallic acid curve plotted using different concentrations of gallic acid (50, 100, 150, 200, 250, 300, 350, 400 and 450 µg/mL) in 5.0 mL of Folin Ciocalteu reagent (diluted tenfold) and 4.0 mL of sodium carbonate solution (75 g/L) was used for comparative assessment. Thus, the content obtained was mentioned as gallic acid equivalent (GAE) in µg/mL of the dry extracts.

Total flavonoid content determination
Sample solution (1 mg/mL; 5 mL) of the extract was mixed with equal volume of aluminium trichloride (2%). Contents were kept aside for about 10 min and using 415 nm, the absorbance was measured. Five mL of methanol extract solution without aluminium trichloride was used as control. Standard curve of quercetin (10–100 µg/mL) was employed to determine total flavonoid content and the average of 3 readings was taken and expressed as QE, that is, Quercetin equivalent, on dry weight basis.

Antioxidant assays
DPPH radical scavenging assay
Spectrophotometric method was employed to measure DPPH scavenging activity. To 0.05 mL of alcoholic, aqueous extracts (10-140 µg/mL) of BC and standard ascorbic acid in ethanol, added an 0.05 mL of ethanolic solution of DPPH (200 µM). Equal volume of ethanol was used as blank. DPPH free radicals quenching was observed and measured at 517 nm after 20 min following which inhibition percentage was calculated as given below;

\[ \% \text{ inhibition} = \frac{(\text{control} - \text{test})}{\text{control}} \times 100 \]

ABTS radical cation depolarization assay
ABTS solution (7 mM) in presence of ammonium persulfate (2.45 mM) generates ABTS\(^{+}\) radical cations. The reaction mixture was kept aside at room temperature overnight. 0.5 mL of alcoholic, aqueous extract (10-140 µg/mL) and standard ascorbic acid were then added to ABTS solution (0.3 mL). One mL ethanol was used to make up the final volume. Percentage inhibition was calculated after measuring the absorbance at 745 nm.
Assay of Nitric oxide radical scavenging activity
Nitric oxide generated through sodium nitroprusside was measured using Griess’ reaction at the temperature conditions maintained at 25°C for 5 h. Sodium nitroprusside (5 mM) (in standard phosphate buffer solution) was incubated with various concentrations (25-400 µg/mL) of ethanolic, aqueous extracts and standard ascorbic acid in phosphate buffer of 0.025 M and pH: 7.4. Similarly, control experiment was carried out by omitting the test compound which was replaced with equal volume of buffer. Then, 0.5 mL of incubated solution was mixed with 0.5 mL of Griess’ reagent (0.1% naphthyl-ethylene-diamine-dihydrochloride and 1% sulphanilamide and 2% O-phosphoric acid). The color developed through diazotization and coupling of nitrite with sulphanilamide and naphthyl-ethylene-diamine respectively, was read at 546 nm.

Superoxide radical scavenging assay
Alkaline DMSO method was used to perform the potassium superoxide scavenging activity. Dry DMSO and Potassium superoxide mixture were kept in contact for a day. Filtration of the particular solution was ensured before it was used. Two hundred µL of the filtrate was pipetted into 2.8 mL aqueous solution having 56 µM NBT, potassium phosphate buffer (10 mM) and EDTA (10 µM). At 560 nm, absorbance was recorded for extracts and standard (ascorbic acid) at different concentrations (20-140 µg/mL) by using pure control DMSO. Inhibition (%)=[(Control–Test)/Control] x 100

Hydrogen peroxide scavenging assay
To 40 mM of H2O2 solution (0.6 mL) in phosphate buffer with pH 7.4 extract samples (100 µg/mL in distilled water) was added. Ten min later using 230 nm the absorbance of above mixture was read. A blank without hydrogen peroxide in the buffer solution was prepared. The percentage scavenging capacity of H2O2 by both BC extracts and the standard (ascorbic acid) were obtained using the below formula.

% Scavenged [H2O2] = [(A0 – A1) / A0] x100

Animals
Healthy Wistar albino rats weighing between 200–250 g for the study were obtained from Central Animal Research Facility of MAHE, Manipal, Karnataka. Polypropylene cages were used to house the animals and standard conditions were maintained throughout (12 h light: 12 h dark cycle, temperature range of 25±3°C and humidity ranging between 35–60%). Animal feed included standard rat pellet diet which was procured from VRK Nutritional Laboratory Animal Feed, Maharashtra, India and water ad libitum. Approval for conducting the present work was obtained from KMC, Manipal, India and the reference No. is IAEC/KMC/112/2013.

Antidiarrheal activity
Diarrhea-induced by administering castor oil in rats
Orally administered castor oil in the dose of 3 mL/kg, po has induced diarrhea in rats. The experimental animals were not fed for 16 h. During this fasting time, rats were allowed to drink sufficient water. Before the commencement of the experiment, arbitrarily animals were distributed into six groups and each group having 6 rats (36 animals). Following this, each rat was then placed separately in polypropylene cages having transparent sheet at its bottom. All the rats of Group I were given 10 mL/kg oral administration of propylene glycol which served as vehicle control. Group II served as the disease control and received Castor (3 mL) oil alone. Rats from the Groups III, IV and V were given 10 mL/kg oral administration of BCAL 100, 150 and 200 mg/kg as test doses respectively. Group VI rats were orally administered with the standard drug Loperamide in the dose of 10 mg/kg. After 30 min of pretreatment, all the rats were given castor oil in the dose of 3 mL/kg orally. Rats were monitored for their diarrheal droppings for 6 h. Factors like total number of fecal output, time elapsed between castor oil administration and the first instance of diarrheic fecal excretion were noted. The other factors including total diarrheic fecal weight and its weight particularly in that period were also recorded. Each animal’s excreted diarrhea stool was numbered and calculated in the said period. The diarrhea induced after castor oil administration and its severity in rats were also noted and scored. Dicarlo et al. (1994) method of numerical score which on the basis of stool consistency was given as normal stool (or without diarrhea) = 1, semisolid = 2, and 3 for watery stools/ feces respectively. Correspondingly, the percentage and purging in dices (through comparison with control group) were calculated. Interval of time (min) between castor oil administration and the first diarrheic stool appearance was considered for the measurement of onset of diarrhea. All the fecal matter that was collected during 6 h period was weighed. Body
weight differences at pre as well as post treatment periods were noted down. Calculation of percentage score defecation inhibition and weight of the feces was done by\textsuperscript{17} the following formula.

\[
\frac{\text{control mean - treated (test) mean}}{\text{control mean}} \times 100
\]

Enteropooling, intestinal transit time and fluid accumulation in the intestine induced by castor oil administration

All rats from various groups under examination were not given food for 16 h. However, the rats had free access to sufficient drinking water during this time period. Then, the animals were arbitrarily allocated into six groups of six each. After grouping, rats were then placed separately in polypropylene cages having transparent sheet at the bottom. Group I rats were given 10 mL/kg (\textit{p.o.}) of propylene glycol (in which loperamide and BCAL were dissolved). Group II was administered Castor oil only. Rats from Group III, IV and V received orally 10 mL/kg of BCAL at 100, 150 and 200 mg/kg, correspondingly. Following this, castor oil (3 mL/kg) was administered orally. Loperamide, at 10 mg/kg (\textit{p.o.}) was given to all the rats of group VI and followed by castor oil (3 mL/kg) administered orally. After thirty minutes, physiological saline containing activated charcoal (10\%, 3 mL/kg) was administered orally to all the rats after forty minutes of charcoal meal. The abdomen was cut open. Ligation through the entire intestine from the pylorus to caecum carried out followed by dissection and removal. In the small intestine, charcoal meal has traveled certain length which was determined indicating the Peristaltic index (PI) which was later expressed in terms of percentage of small intestine’s total length. The whole intestine was weighed. A graduated measuring cylinder was taken in which all the intestinal contents were expelled and the volume was measured. Weight variation between full and empty intestine were noted. For every 10 mg/kg oral dose of the standard BCAL at 100, 150 and 200 mg/kg, \textit{p.o.} showed definite percentage inhibition of movement of charcoal meal in the intestine. It was calculated as a negative control (vehicle treated group) by the use of following formula.

\[
\% \text{ transit inhibition} = \left[\frac{T_o - T_t}{T_o}\right] \times 100
\]

Where,

To stands for mean length which the charcoal meal traverses in the rats of vehicle-treated group;
Tt is the mean length where charcoal meal transverses in rats treated with standard loperamide and BCAL test groups\textsuperscript{17}.

Acute toxicity studies

OECD 425 guidelines were followed to check for acute toxicity with a ceiling dose of 2000 mg/kg of BC. In brief, the rats were assessed every 10 min for a period of 4 h. They were also assessed for 24, 48, and 72 h to check for any kind of changes in respiration, piloerection, motor activity (spontaneous), gait and writhing once the drug was administered. The follow up process of animals went up to 30 days for mortality.

Statistical analysis

The statistical significance between the groups was determined by the one-way ANOVA (analysis of variance) followed by the post-hoc Dunnett’s test using Prism 5.03 Demo Version (Graph Pad Software Inc., La Jolla, CA). The minimum level of significance was set at \(p<0.001\). All values were expressed as mean standard error of mean (SEM), n=6 (unless otherwise specified).

Results

The percentage yield of BCAL and BCAQ were 28.93 and 14.90 mg/g, respectively. The total phenolic content for BCAL and BCAQ was found to be 24.60 and 31.71 mg/g, respectively calculated as gallic acid equivalent (GAE). The flavonoid content in total of BCAL and BCAQ was 2.503 and 2.501 mg/g, respectively calculated as quercetin equivalent (QE).

IC\textsubscript{50} values were determined for the different models and are represented in Table 1. Ethanolic

<table>
<thead>
<tr>
<th>Sample</th>
<th>DPPH assay</th>
<th>ABTS assay</th>
<th>Nitric oxide assay</th>
<th>Superoxide assay</th>
<th>H\textsubscript{2}O\textsubscript{2} assay</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standard</td>
<td>0.97±0.04</td>
<td>8.81±0.56</td>
<td>28.62±1.89</td>
<td>11.38±0.98</td>
<td>21.96±2.03</td>
</tr>
<tr>
<td>BCAL</td>
<td>5.50±0.42*</td>
<td>7.94±0.64\textsuperscript{ns}</td>
<td>25.99±3.62\textsuperscript{ns}</td>
<td>13.54±0.84\textsuperscript{ns}</td>
<td>18.10±1.08\textsuperscript{ns}</td>
</tr>
<tr>
<td>BCAQ</td>
<td>10.97±1.02*</td>
<td>9.18±0.66\textsuperscript{ns}</td>
<td>25.28±2.44\textsuperscript{ns}</td>
<td>16.45±1.24*</td>
<td>22.57±4.82\textsuperscript{ns}</td>
</tr>
</tbody>
</table>

All values are expressed as mean±S.D., n=6. *\(p<0.05\) vs. respective standard; ns – no significant difference vs. standard by one-way ANOVA with Dunnett’s post hoc test.
extract of churna showed more effective results for different assays compared to aqueous extract.

**Acute toxicity**
Acute toxicity studies were performed for 2000 mg/kg, all animals survived and showed no abnormal behavior or signs of toxicity. For further studies, the doses selected were between 1/10th and 1/20th of the tolerated dose in rats.

**Antidiarrheal activity**

**Diarrhea in rats induced by treatment with castor oil**
The control group rats exhibited copious diarrhea around two-and-half hours post oral castor oil administration. The onset of diarrhea was significantly delayed in rats pretreated with BCAL (100, 150 and 200 mg/kg, po). It also had an impact on the frequency and reduced the number of times of defecation as well as the fecal wetness (decrease in the number of stools which are wet and in total). Loperamide, the standard drug (10 mg/kg, po), exhibited a greater inhibition on diarrheal parameters under examination compared to BCAL (Table 2).

**Intestinal transit in rats induced by castor oil administration**
In control rats, 95% transit of charcoal along the length of the intestine was observed (Table 3) after 0.5 h of gastric administration. In the BCAL administered group of rats, (100, 150, 200 mg/kg, po) a significant (p<0.001) decrease in propulsive motion and charcoal meal transit was observed. Loperamide (10 mg/kg, po) exhibited higher effect of anti-motility in comparison to maximum dose of BCAL.

**Enteropooling in rats induced by castor oil**
A statistically significant (p<0.001) and marked surge in the volume of intestinal fluid was observed on oral administration of castor oil 3 mL/kg to rats in comparison to vehicle control group which was treated with propylene glycol (10 mL/kg, po) alone. Prior treatment of the test group rats with BCAL (100, 150 and 200 mg/kg, po) inhibited fluid accumulation that was induced by castor oil and the effect was significant (p<0.001) in comparison with the control group (Table 4). It was also observed that loperamide (10 mg/kg, po) demonstrated a higher significant inhibitory effect (p<0.001) on fluid accumulation induced by castor oil as compared to the maximum dose of BCAL (100, 150 and 200 mg/kg, po).

**Discussion**
Balacaturbhadrak churna is an Ayurvedic pediatric preparation used for various GIT diseases since ages. The botanical ingredients of the churna prompted us to validate the claimed activity biologically and relate the activity to possible mechanistic frame. It was therefore aimed at determination of phenolic and flavonoid content of the churna; estimation of antioxidant activity and evaluation of Antidiarrheal activity in the animals. Oxidative damage is assumed to be the root cause of many diseases, we tested various extracts for possible antioxidant potential of the product.

Redox property of phenolic compounds and their activity is facilitated by hydroxyl groups, and thus act

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### Table 2 — Effects of BCAL (100-200 mg/kg, po) on castor oil-induced diarrhea in rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mL/kg) or (mg/kg)</th>
<th>Total no. of stools</th>
<th>No. of wet stools</th>
<th>% protected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>0.00±0.00</td>
<td>0.00±0.00</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>7.33±0.33</td>
<td>6.50±0.42</td>
<td>0</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>6.50±0.22***</td>
<td>0.16±0.16***</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>5.50±0.34***</td>
<td>0.33±0.21***</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>4.83±0.30***</td>
<td>0.16±0.16***</td>
<td>34</td>
</tr>
<tr>
<td>Loperamide</td>
<td>10</td>
<td>0.00±0.00***</td>
<td>0.00±0.00***</td>
<td>100</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M., n = 6. ***p<0.001 vs. control by one-way ANOVA with Dunnett’s post hoc test.

### Table 3 — Effects of BCAL (100-200 mg/kg, po) on castor oil-induced intestinal transit in rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mL/kg) or (mg/kg)</th>
<th>Peristaltic index (%)</th>
<th>Inhibition of transit (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>0.00±0.00</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>95.10±0.42</td>
<td>0</td>
</tr>
<tr>
<td>Extract</td>
<td>100</td>
<td>59.22±3.14***</td>
<td>42</td>
</tr>
<tr>
<td></td>
<td>150</td>
<td>31.75±1.10***</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.00±0.00***</td>
<td>100</td>
</tr>
<tr>
<td>Loperamide</td>
<td>10</td>
<td>0.00±0.00***</td>
<td>100</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M., n = 6. ***p<0.001 vs. Control by one-way ANOVA with Dunnett’s post hoc test.

### Table 4 Effects of BCAL (100-200 mg/kg po) on castor oil induced enteropooling and fluid accumulation in rats

<table>
<thead>
<tr>
<th>Treatment group</th>
<th>Dose (mL/kg)</th>
<th>Intestinal fluid (mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>10</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>Control</td>
<td>10</td>
<td>1.05±0.04</td>
</tr>
<tr>
<td>Extract</td>
<td>150</td>
<td>0.71±0.03***</td>
</tr>
<tr>
<td></td>
<td>200</td>
<td>0.23±0.04***</td>
</tr>
<tr>
<td>Loperamide</td>
<td>10</td>
<td>0.45±0.06***</td>
</tr>
</tbody>
</table>

All values are expressed as mean ± S.E.M., n = 6. ***p<0.001 vs. Control by one-way ANOVA with Dunnett’s post hoc test.
As antioxidants. Free hydroxyl groups particularly
3-OH, plant flavonoids possess antioxidant
properties, as known antioxidants and have been validated for
their activity by various researchers. Phenolic and
flavonoid compounds from the rhizomes of *Cyperus rotundus*—an important ingredient of BC, were found
to be methyl 3,4-dihydroxy benzoate and rutin. Rutin is a common antioxidant used as standard for
antioxidant activity. The galls of *Pistacia integerrima*
(Karkatasringi), are celebrated antioxidant agents.
14′-phenoxytetradecany 3,5-dihydroxy benzoate
(pistipholo-glucinyl ester) 2, 4′-phenoxy-n-butyl-1′-
(3oxy-5-hydroxy) benzoic acid (pistacaphenyl ether) 3
and 3′-(1,3-dihydroxy-5-phenoxy-1′,5′-dimethoxy-
benzene (pistipholo-glucinyl ether) have been
reported from the galls and all of them are known
antioxidants. Several studies have been undertaken
to justify the use of Pippali as an antioxidant. Thus, the antioxidant activity possessed by BC
extracts can be attributed to its flavonoid and the total
phenolic contents.

Antioxidants have been advised in many diseases.
The implication of antioxidants had also been
reported in diarrhea. Hu et al. have reported the
application of phenols and flavonoids in diarrhea and
have carried out their antioxidant activity.

The DPPH is a stable free radical that is widely
used in assessing free radical scavenging activity of
the plants extracts rich in secondary metabolites, like
phenolics and flavonoids. In our study, the alcoholic
extract of the churna (BCAL) showed better
antioxidant activity when compared to aqueous
extract BCAQ in DPPH free radical scavenging assay.

Inhibition of absorbance of ABTS radical cation
takes place in ABTS assay and is characterized by
long wavelength absorption spectrum. From the result
obtained, it can be indicated that the activity of churna
extracts is either by inhibiting or by scavenging
property as an antioxidant activity towards ABTS free
radical.

The unstable radical nitric oxide will react with
oxygen to produce the intermediates NO₂, N₂O₄ and
N₂O₃ and eventually stable product nitrate and nitrite.
Griess reagent is used to estimate this. In our
investigation, the extracts reduced the nitrite that was
formed by incubating sodium nitroprusside in
standard phosphate saline buffer at 25°C. It may be
due to extract constituents which show antioxidant
activity competing with oxygen in order to react
with nitric oxide and thus not allowing the formation
of nitrite.

Superoxide dismutase acts as the catalyst for
dismutation of highly reactive superoxide anion to
H₂O₂ as well as oxygen. The first reduction product of
oxygen is superoxide anion which is measured in terms
of inhibited generation of O₂. In the present study, on
*in vitro* reaction mixture, extracts showed dose
dependent inhibition of generation of superoxide.

Hydrogen peroxide plays a vital role in the pathogenesis of various diseases. Under physiological
pH and temperature, it is very stable and has the
capacity to penetrate the membrane. Because of its
weak oxidizing ability and relatively lesser toxicity, it
is still regarded as less reactive. Furthermore,
cellular damages are mediated by the hydroxyl radical
which is the conversion product in presence of transition metal. Singlet oxygen may also be formed
by reacting with hypochlorous acid (HOCI) or
superoxide anion or chloramines in living system.
Thus, while assessing the anti-oxidant activity,
measurement of H₂O₂ scavenging activity becomes an
important tool. In our study, BCAL produced better
protection against H₂O₂ compared to BCAQ. The
antioxidant results from different assay methods
demonstrated coherent results suggesting better
activity of BCAL when compared to BCAQ.
Therefore, in the present study, BCAL was selected
for the *in vivo* animal model.

Castor oil induced diarrhea being most widely used
animal model for antidiarrheal activity and therefore,
it was selected for the present investigation. Castor oil
stimulates diarrhea by three different mechanisms
namely-(1) releasing nitric oxide and thereby
increasing permeability of gastrointestinal membrane
for calcium; (2) stimulating prostaglandin synthesis
and thereby increasing fluid and electrolytes into
the lumen of the bowel; and (3) increasing peristalsis.
Our results showed sharp increase in number of stools
after administration of Castor oil indicating diarrhea.
An appreciably high peristaltic index was also
observed in the untreated control. High intestinal
enteropooling was also noticed. From the above
observations, induction of diarrhea was confirmed.
BCAL Extracts having shown good activity were
given at three different doses to study the effect of the
extract on diarrhea. At a lower dose (100 mg/kg) the
extract showed very mild activity and lower protection against castor oil induced diarrhea. At the middle and higher doses (150 and 200 mg/kg respectively) the extract exhibited significant protection against castor oil assault (p<0.001 in both the cases). The elevated total number of stools due to castor oil was reduced to 4.83 from 7.33, indicating 34% protection, though, the complete reduction in wet stools indicated complete reduction in diarrhea. However, the churna extract did not stop the stools completely further leading to forced constipation. Thus, Balacaturbhadrika churna is a better option when compared to loperamide. Both Churna extracts and loperamide have successfully prevented water loss and improved peristaltic index.

The underlying mechanism in the protection against castor oil induced diarrhea could be attributed to the phenolic and flavonoid contents of the churna. In some of the previous studies, Tannins and Flavonoids have been reported to possess anti diarrheal activity. The phenolics and flavonoids present in BC are thought to be responsible for the anti diarrheal activity. The phenols and flavonoids are known to increase colonic water and electrolyte reabsorption and thus act by inhibiting intestinal motility.

Conclusion

The current experiment showed substantial amounts of flavonoids and phenolics in churna extracts. The potential antioxidant activity displayed by BC extracts could be attributed to these contents. Thus, churna extracts can be used to treat the diseases mediated by free radicals. However, BCAL showed better activity in most of the models. The experimental proof or evidence generated from the current study indicates BCAL to possesses anti diarrheal activity in rats. These results could pave way towards lending pharmacological basis to or confirm the usage of BC in traditional medicine for managing and controlling diarrhea in children.

Abbreviations used

BC: Balacaturbhadrika churna
BCAL: Balacaturbhadrika churna alcoholic extract
ACAO: Balacaturbhadrika churna alcoholic extract
ABTS: 2,2-azinobis-(3-ethylbenthiazoline-6-sulphonate)
DPPH: 1,1-Diphenyl-2-picryl-hydrazyl
OECD: The Organization for Economic Co-operation and Development
IAEC: Institutional Animal Ethics Committee
KMC: Kasturba Medical College

References

15. Henry LEA, Halliwell B, Hall DO The superoxide dismutase activity of various photosynthetic organisms measured by a


