Bio-extract Concentrated of Thai “Yor” *Morinda citrifolia* Effects in Analgesic, Acute Toxicity and Human Peripheral Blood Mononuclear Cells

Pranee Nandhasri*, Sopit Thamaree**, Tadsanee Punjanon***, Somboon Kietinun*

**Abstract**

BCTX, Bio-extract concentrated Thai “Yor” or Noni in English, *Morinda citrifolia* L. (Rubiaceae), was found the total fatty acids and amino acids content of $149 \pm 8.74$ and $204 \text{mg/100 g}$, respectively. It was reported to be a nontoxic bio-extract with the acute toxicity (LD$_{50}$) of more than $4,000 \text{mg/kg body weight}$ of Swiss albino mice and Wistar rat. The methyl alcohol extract of *M. citrifolia* raw material from BCTY product revealed the analgesic effect of $93.1 \pm 1.7\%$ inhibition which was quite equivalent to $96.7 \pm 1.6\%$ of morphine sulfate $1.5 \text{mg/kg}$. Human peripheral blood mononuclear cells (HPBMCs) were used for cytotoxicity, CD$_4$/CD$_8$ counts. The BCTY extract $500 \mu\text{g/ml}$ increased in cell viability $160.29 \pm 1.35\%$ (compared to $100\%$ of the control) and stimulated lymphocyte proliferation $84.04 \pm 10.42\%$ while the CD$_4$ and CD$_8$ were $107.83 \pm 3.21\%$ and $98.00 \pm 2.74\%$, respectively.

**Key words:** Yor, Noni, *M. citrifolia* L. (Rubiaceae), immune activity, nontoxic bio-extract, human peripheral blood mononuclear cells (HPBMCs), cytotoxicity

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Introduction

Over the last decade, a growing number of people have become interested in the medicinal uses of noni juice, made from the fruit of the Indian mulberry, *Morinda citrifolia*, L. (Rubiaceae) of the South Pacific Islands of Tahiti, and more recently from Hawaii. *Morinda citrifolia* has been used in folk remedies by Polynesians for over 2000 years. Yor in Thailand and Hawaii noni are found to be the same cultivars by DNA fingerprints with the AFLP technique. More than 120 nutraceutical compounds identified in Noni are reported to have a broad range of therapeutic effect of alkaloid xeronine essential to maintain normal function of all cells. The other therapeutic effects of noni include antibacterial, antiviral, antifungal, antitumor, antihistamine, analgesic, hypotensive, anti-inflammatory and immune enhancing effects, which have relieved 15,000 peoples suffering from 23 conditions. Noni juice is also reported to exhibit activities against various kinds of microorganisms such as *Pseudomonas aeruginosa*, *Bacillus subtilis*, *Escherichia coli*, *Streptococcus pyogene* and *Salmonella shigella*.

The bio-active concentrated of Thai Yor (BCTY) juice or bio-active Yor juice (BYJ) was produced from *Morinda citrifolia* (name in Thai “Yor”) typically grown in Thailand. Using TLC-method, Phytochemicals including alkaloids, anthraquinones, antioxidants, essential oils, flavonoids, saponins and scopoletin have also been shown in the paper of Nandhasri et al. The BCTY product has been shown no contamination of lead (Pb), microorganisms (e.g. *E. coli*, yeast, mold, coliform, *C. perfringens*, *S. aureus*, *B. cereus*), and no synthetic food preservative (e.g. synthetic color, sodium benzoate and benzoic acid.).

Acute toxicity test was performed in both male and female (n = 10/group) of Wistar rats (weight 200 - 250 g) and Swiss albino mice (weight 25 - 30 g). A maximum dose of BCTY juice (10 ml/kg), was administered by gavage for rodent. The animals were observed for 14 days following treatment. The results of acute toxicity showed that all animal survived and no adverse clinical signs were noted for 14 days following treatment. Organ necropsy revealed no signs of gross toxicity. The recent study of Ratanavalachai et al., found that Thai Noni fruit juice or the BCTY juice have no genotoxic against human lymphocytes in vitro. In addition, pretreatment of Noni fruit juice at 6.2 mg/ml demonstrated no anticlastogenic effect while had some antigenotoxic effects as demonstrated by significant decrease in the sister chromatid exchange level induced by mitomycin C (p < 0.05).

More scientific supports are still needed for the consuming of this Thai yor fruits as a juice product. This paper reported on the quality control data in physical and chemical properties of the *Morinda citrifolia* bio-active juice product such as amino acids, fatty acids, acute toxicity, analgesic effect, immunity (cytotoxicity, stimulation and CD4/CD8 counts) and stability test of the product.

2. Materials and Methods

2.1 Bio-extract concentrated of Thai “Yor” (BCTY)

Yor (*Morinda citrifolia* L.) fruits were grown and obtained from Nakorn Rajasima province from the north eastern part of Thailand. Bio-extract concentrated Thai Yor (BCTY) juice was prepared from well-clean of fresh-matured *M. citrifolia* fruits mixing with red cane sugar in the portion of 3 : 1 in the same procedure as Nandhasri et al. The mixture was often stirred day by day during the first 2 weeks then left the reactor at room temperature (about 37 °C) until the foul smell of Yor disappeared, with in about 3 - 6 months. The BCTY juice was filtered through clean-boiled cotton cloth.
2.2 Physical, chemical and chromatographic method

Gas chromatography method was used for determining fatty acids according to Pendl et al.\textsuperscript{10} and liquid chromatography (LC) for amino acids analysis\textsuperscript{11} of BCTY. Protein content\textsuperscript{11} was run for the stability test of BCTY juice during the days of 391 - 491.

2.3 Animals

Male Swiss mice weighting 30 - 35 g were obtained from National Laboratory Animal Center, Mahidol University, Thailand. The experimental protocol was approved by the Institutional Animal Ethics Committee. The animals were housed at 22 ± 2 °C temperature under 12-h light/12-h dark cycle and with access to food and water ad libitum. Before experiments began, the animals were deprived of food for 24 h and allowed to adapt to the laboratory for at least 2 h before testing.

2.4 Analgesic effect determination: Acid-Induced Abdominal Constriction (Writhing) Test in Mice

The alcoholic extract of the Thai Yor (\textit{Morinda citrifolia} L.) fruits, which prepared from BCTY juice, was evaluated for analgesic effect in mice using the classical method, acetic-induced writhing response.\textsuperscript{12,13} Treating the mice prior to testing, each animal received the alcoholic extract at dose of 1, 2, 3 and 4 g kg\textsuperscript{-1}, i.p. Control animals received normal saline (10 ml kg\textsuperscript{-1}, i.p.) instead of the extract. Morphine sulphate (Merck, AG, Armstadt, Germany), 1.5 mg kg\textsuperscript{-1}, i.p. was used as a reference analgesic substance. Fifteen minutes after treatment, the mice were injected with 0.75% (v/v) acetic acid (Sigma, St. Louis, USA) in normal saline (10 ml kg\textsuperscript{-1}, i.p.); the number of induced writhings (arching of back, development of tension in abdominal muscles, elongation of the body in hind limb) were recorded for a period of 20 minutes.

For evaluation of time-course analgesic effect, the number of induced writhings of the mice with alcoholic extract injection of \textit{Morinda citrifolia} (4 g kg\textsuperscript{-1}, i.p.) were recorded at 15, 60, 120, 180, 300 and 480 minutes.

2.5 Human peripheral blood mononuclear cells (PBMCs) preparation

The blood for the preparation of human peripheral blood mononuclear cells (PBMCs) were obtained from healthy male blood donors, aged between 20 - 35 years, at the National Blood Bank, Thai Red Cross Society. PBMCs ($1 \times 10^6$ cells/ml) isolated by Ficoll–Hypaque gradient centrifugation, were counted and adjusted for culturing in 96-well microtiter plate with the \textit{Morinda citrifolia} extract to have the appropriate concentration of 4, 20, 100 and 500 µg/ml in complete RPMI-1640 medium. The cultured \textit{M. citrifolia}-PBCMCs was incubated at 37 °C under 5% CO$_2$ for 72 hours. This cultured \textit{M. citrifolia}-PBCMCs were used for cytotoxicity assay, lymphocyte proliferal assay and CD$_4$/CD$_8$ counts.

2.6 Cytotoxicity assay

The cultured \textit{M. citrifolia}-PBCMCs was run for cytotoxicity by MTT assay in determining the percent of cell viability.\textsuperscript{14} The reduction of yellow MTT [3-(4,5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide, a tetrazole] of Sigma, St. Louis, USA] in this assay, takes place only when mitochondrial reductase enzymes are active, is reduced to purple formazan. The absorbance of purple formazan solution can be quantified by a spectrophotometric method of the microplate reader at 570 nm and 650 nm.

Percent of cell viability = \[\frac{OD of treated cells}{OD of control cells}\times 100.\]
2.7 Lymphocyte proliferal assay

Eighteen hours before the end of M. citrifolia-PBCMCs cultured procedure, 25 µl of [³H]-thymidine (20 µCi in RPMI-1640 medium) was added into each well. Harvested the cells on glass fiber filters using a cell harvester. The radioactivity was measured as count per min (CPM) by β-scintillation counter and calculated the percentage of stimulation:

\[
\% \text{ stimulation} = \left[ \left( \frac{\text{CPM (sample)} - \text{CPM (control)}}{\text{CPM (control)}} \right) \times 100 \right]
\]

2.8 CD₄/CD₈ counts

Carefully removed the supernatant from the cultured M. citrifolia-PBCMCs and pipetted the cells (50 µl) from the well into the tube. Then, added 5 µl of anti CD₄-FITC (fluorescein isothiocyanate, CD₈-PE (phycoerythrin) and CD₃-perCP (peridinin chlorophyll protein) in to each tube for CD₄, CD₈ and control, respectively. Mixed and incubated in the dark for 20 minutes at room temperature. Thereafter, added 2 ml of washing buffer PBS into each tube and centrifuged at 1,500 rpm for 5 minutes at 4 °C. removed washing buffer and added 200 µl of 0.5% formaldeyde and detected CD₄, CD₈ and CD₃ (control) on cultured M. citrifolia-PBCMCs by using Flow cytometer.

SEB (Staphylococcal enterotoxin B) was used as the positive control.

2.9 Statistical analysis

Results are expressed as the mean ± S.M.S. of n = 3 for fatty acid not considered the p-value (Table 3). Number of writhes were expressed as mean ± S.E.M. and % protection (i.e., compound producing 100% protection prevents acetic acid-induced abdominal constriction) which was calculated using the formula:

\[
\% \text{ Protection} = 100 - \frac{(\text{number of writhes in drug treated} \times 100)}{(\text{number of writhes in control})}
\]

Statistical analysis of the data was done using Student’s t-test and the results were considered significant when p < 0.01, n = 6 for analgesic effect (Table 4 and Figure 1). For experiments of % cell viability and % stimulation, n = 5 (Table 5) and CD₄/CD₈ counts (Table 6), the p value ≤ 0.05 were considered to be significant different when compared with control.

3. Results

3.1 Total fatty acids, amino acids and protein determination for the stability of BCTY juice products.

Total fatty acid content in BCTY was 149 ± 8.735 mg/100g with the major component of C14 : 0 (107 ± 8.7 mg/100 g). (Table 1). The content of 9 essential amino acids in BCTY was 63 mg/100 g in the total content of 17 amino acids of 204.0 mg/100 g. The content of these amino acids was summarized as follows: Glu, Ala, Arg (range 20 - 26 mg/100 g); Gly, Cys, Met, Tyr, Phe, Lys (range 9.0 - 14.5 mg/100 g) and Thr, Ser, Val, Ile, Leu, Try (range 3 - 6.5 mg/100 g). The highest and lowest content was Asp 34.9 (mg/100 g) and His (2.0 mg/100 g), respectively (Table 2). The protein determination showed that the stability of BCTY juice products and dried M. citrifolia powder was 451 days (Table 3).
3.2 Analgesic of the alcoholic extract of *M. citrifolia* on acetic acid–induced writhing in mice.

In control mice, the number of writh during the 20 min test period was 43.0 ± 1.4 (n = 6). Analgesic effect of the alcoholic extract of *M. citrifolia* at a dose of 4,000 mg Kg⁻¹ on acetic acid–induced writhing in mice demonstrated i.p. inhibition of 93.1 ± 1.7%, which was similar to morphine sulphate in which its dose of 1.5 mg/Kg exhibited i.p. inhibition of 96.7 ± 1.6% (Table 4). The time-course analgesic effect evaluation of the alcoholic extract of *M. citrifolia* at a dose of 4,000 mg Kg⁻¹ was statistically significant (p ≤ 0.001) for 15 min until 5 hr of pretreatment time (Fig.1). Administration of the alcoholic extract of *M. citrifolia* at a dose of 1,000 to 4,000 mg Kg⁻¹ did not produce mortality in mice.

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**Table 1** Fatty acids (FA) content (mg/100 g) of Bio-extract Concentrated of Thai Yor, *M. citrifolia* (BCTY)

<table>
<thead>
<tr>
<th>Product</th>
<th>C4</th>
<th>C6</th>
<th>C8</th>
<th>C10</th>
<th>C14</th>
<th>C16:1</th>
<th>Total FA</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCTY (N=3)</td>
<td>18 ± 8</td>
<td>10 ± 3</td>
<td>10 ± 3</td>
<td>3 ± 5</td>
<td>107 ± 8.7</td>
<td>1 ± 2</td>
<td>149 ± 8.76</td>
</tr>
<tr>
<td>NJ-S-Pacific*</td>
<td>10</td>
<td>0</td>
<td>10</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>NJ-Tahiti*</td>
<td>0</td>
<td>0</td>
<td>20</td>
<td>0</td>
<td>120</td>
<td>0</td>
<td>140</td>
</tr>
</tbody>
</table>

* Noni juice of South Pacific (NJ-S-Pacific) and Noni juice from Tahiti (NJ-Tahiti) are the commercial product from USA: South Pacific and Tahiti, respectively.

**Table 2** Amino acids content (mg/100g) of Bio-extract Concentrated of Thai Yor, *M. citrifolia* (BCTY)

<table>
<thead>
<tr>
<th></th>
<th>Asp</th>
<th>Thr</th>
<th>Ser</th>
<th>Glu</th>
<th>Gly</th>
<th>Ala</th>
<th>Cys</th>
<th>Val</th>
<th>Met</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>34.90</td>
<td>6.0</td>
<td>5.0</td>
<td>26.0</td>
<td>9.0</td>
<td>24.0</td>
<td>14.5</td>
<td>6.5</td>
<td>10.0</td>
</tr>
<tr>
<td>Ile</td>
<td>3.0</td>
<td>5.1</td>
<td>9.5</td>
<td>14.0</td>
<td>9.0</td>
<td>2.0</td>
<td>5.5</td>
<td>20.0</td>
<td>204</td>
</tr>
</tbody>
</table>

**Table 3** Percent protein determination for stability of Bio-extract Concentrated of Thai Yor, *M. citrifolia* (BCTY) compared to dried fruit mixed of *M. citrifolia* (M.C.)

<table>
<thead>
<tr>
<th>Product</th>
<th>% Protein, determination at time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>391 days</td>
</tr>
<tr>
<td>Dried fruit mixed (M.C.)</td>
<td>7.71</td>
</tr>
<tr>
<td>BCTY No. 3</td>
<td>0.75</td>
</tr>
<tr>
<td>BCTY-5 blend</td>
<td>0.85</td>
</tr>
</tbody>
</table>
Table 4  Analgesic effect of the alcoholic extract of bio-extract concentrated of Thai Yor, M. citrifolia (BCTY) on acetic acid-induced writhing in mice

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg Kg⁻¹), i.p.</th>
<th>No. of writhes²</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (NSS, 10 ml Kg⁻¹)</td>
<td>-</td>
<td>43.0 ± 1.4</td>
<td>-</td>
</tr>
<tr>
<td>Morphine sulphate</td>
<td>1.5</td>
<td>1.3 ± 0.6*</td>
<td>96.7 ± 1.6</td>
</tr>
<tr>
<td>*p ≤ 0.001 significantly different from control, Student’s t-test.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Morinda citrifolia extract</td>
<td>1,000</td>
<td>41.0 ± 1.9</td>
<td>4.4 ± 4.5</td>
</tr>
<tr>
<td>2,000</td>
<td></td>
<td>33.5 ± 4.6</td>
<td>21 ± 11.2</td>
</tr>
<tr>
<td>4,000</td>
<td></td>
<td>3.0 ± 0.7*</td>
<td>93.1 ± 1.7</td>
</tr>
</tbody>
</table>

1 Fifteen minute after treatment, mice were injected i.p. with 0.75% (v/v) acetic acid 10 ml kg⁻¹; the number of induced writhings was counted for 20 min.

2 Values are mean ± S.E.M. (n = 6)

Fig. 1  Time-course analgesic effect of Morinda citrifolia (4 mg kg⁻¹/kg, i.p.) in acetic acid-induced abdominal constrictions (writhes) in mice. Vertical lines show S.E.M. (n = 6), P < 0.001 as compared to 0 min.
3.3 Immune activity of BCTY

Immune activity of BCTY was demonstrated in cytotoxic effect, lymphocyte proliferation effect and CD<sub>4</sub>/CD<sub>8</sub> effect. The cytotoxic effect of *M. citrifolia* extract was measured and expressed as % cells viability. Percent of cell viability showed that all tested concentrations were not toxic to PBMCs (Table 5). A significant increase in the cell number were detected at BCTY concentrations of 100 µg/ml and 500 µg/ml (*p* ≤ 0.05) compared with the control. The effect of BCTY on stimulating lymphocyte proliferation was shown in Table 5. The extract concentrations of 100 and 500 µg/ml stimulated the proliferation of PMBCs. In comparison with the control, *M. citrifolia* extract concentration of 500 µg/ml stimulated lymphocyte proliferation significantly 84.035 ± 10.419% (*p* ≤ 0.05). However, this dose was much less potent than 10 µg/ml of PHA, which substantially stimulated cell proliferation (1101.789 ± 43.711%).

3.4 The percentage of CD<sub>4</sub> and CD<sub>8</sub> count

As shown in Table 6, the percentage of CD<sub>4</sub> and CD<sub>8</sub> count was evaluated by determining CD<sub>4</sub> and CD<sub>8</sub> cells using Flow cytometer. All concentrations of the *M. citrifolia* extract slightly increased the percentage of CD<sub>4</sub> (102.91 ± 2.92% to 107.83 ± 3.21%) and CD<sub>8</sub> (98.00 ± 2.74% to 103.78 ± 2.42%) counts when compared with 100% of the control.

Table 5 Percentage of cells viability and % stimulated lymphocyte proliferation after exposure to different concentrations of bio-extract concentrated of Thai Yor, *M. citrifolia* (BCTY)

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>% cell viability (n = 5)</th>
<th>% Stimulation (n = 5)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00 ± 0.00</td>
<td>100.00 ± 0.00</td>
</tr>
<tr>
<td>PHA 10 µg/ml</td>
<td>152.53 ± 1.73*</td>
<td>1101.79 ± 43.71</td>
</tr>
<tr>
<td>4 µg/ml</td>
<td>105.65 ± 4.23</td>
<td>-33.43 ± 15.65</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>112.21 ± 4.25</td>
<td>-17.70 ± 19.94</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>121.32 ± 3.65*</td>
<td>34.70 ± 14.39</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>160.28 ± 1.35*</td>
<td>84.04 ± 10.42*</td>
</tr>
</tbody>
</table>

(*p* ≤ 0.05 compared with control, n = 5)

Table 6 Effect of different concentrations of Bio–extract Concentrated of Thai Yor, *M. citrifolia* (BCTY) on CD<sub>4</sub> and CD<sub>8</sub> expression on T cells

<table>
<thead>
<tr>
<th>Extract concentration</th>
<th>% CD&lt;sub&gt;4&lt;/sub&gt; count*</th>
<th>% CD&lt;sub&gt;8&lt;/sub&gt; count*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>100.00 ± 0.00</td>
<td>0.00 ± 0.00</td>
</tr>
<tr>
<td>SEB 10 µg/ml</td>
<td>136.68 ± 3.78*</td>
<td>125.11 ± 2.62</td>
</tr>
<tr>
<td>4 µg/ml</td>
<td>102.91 ± 2.92</td>
<td>103.78 ± 2.42</td>
</tr>
<tr>
<td>20 µg/ml</td>
<td>103.61 ± 1.93</td>
<td>101.10 ± 3.58</td>
</tr>
<tr>
<td>100 µg/ml</td>
<td>105.08 ± 14.385</td>
<td>103.53 ± 3.91</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>107.83 ± 3.21</td>
<td>98.00 ± 2.74</td>
</tr>
</tbody>
</table>

(*p* ≤ 0.05 compared with control, n = 5)
4. Discussion and conclusion

Total fatty acid content in BCTY was $149 \pm 8.735$ mg/100g with the major component of C14 : 0 ($107 \pm 8.7$ mg/100g) which was higher than the total fatty acid of Noni juice-Tahiti (140 mg/ml) and of the Noni juice S. Pacific (20 mg/ml), (Table 1). Administration of BCTY, bio-active concentrated Thai Yor (M. citrifolia extract) did not induce any mortality in rats up to dose levels of $8 \text{ g/kg}$, i.p. and $16 \text{ g/kg}$, i.p.

In conclusion BCTY juice is a product of Thailand prepared from M. citrifolia. The quality data in this research were shown that it was safe for consuming as food supplements at doses of 30-50 ml/day. Moreover, it was rich in calcium, phosphorous, vitamin C, vitamin B-complex, anti-oxidant and phytochemicals such as alkaloid, saponnin and scopoletin. BCTY contained reasonable content of fatty acids and amino acids. It is nontoxic and free from microorganisms (e.g. E. coli, yeast and mold, coliform, C. perfringens, S. aureus, and B. cereus) and synthetic food preservative, e.g. synthetic color, sodium benzoate and benzoic acid. Safety of BCTY was assessed by acute toxicity test in wistar rats and Swiss albino mice, both male and female. A maximum volume, 10 ml kg$^{-1}$ of BCTY was administered by gavage in one dose for rodent. All animal survived and no adverse clinical sings were noted for 14 days following treatment and no signs of gross toxicity were seen in the organs after necropsy. BCTY showed analgesic effect, immunity (cytotoxicity, stimulation and CD$^4$/CD$^8$ counts) and was stable for longer than 450 days (Table 3). In addition to this report, BCTY juice has been reported to have more beneficial effects on removing stress causing sleeplessness and preventing cancer. Many food supplements in BCTY juice have been addressed to exhibit various therapeutic effects as follows: anthraquinone: anti–virus and anti–HIV; asperuloside: anti-oxidation; $\beta$-sitosterol: lowering blood cholesterol and stimulating immune system; polysaccharide: stimulating immune system; terpenes: stimulating the synthesis of organic substance and cell strengthening in the body. The coumarin, scopoletin: lowering hypotension, anti-bacterial and mold, anti-histamine, anti-inflammatory and decreasing the pain caused by arthritis, functioning together with serotonin in blood platelets. Damnacanthal has been showed in inhibiting the growth of “ras cells”. It is recommended to drink 30–50 ml/day before meal.

Acknowledgements

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References

บททัศนย์

ผลของน้ำผลิตมัลทาชื้อขึ้น, ต่อการระบายปัสสาวะ, ความเป็นพิษในเซลล์ และในนิวคลีอิโอซอล

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BCTY คือ น้ำผลิตอิมมูนโค้ดขึ้นจากลองไซโตซิค (Morinda citrifolia Lin, Rubiaceae)
นิวคลีอิโอซอล หน่วยอย่างต่ำ ๑๕๕ และ ๒๐๕ มิลลิกรัม/๑๐๐ กรัม ตามลำดับ BCTY ไม่มีความเป็นพิษในเซลล์และ
พบว่าเมื่อใช้ BCTY ในปริมาณ ๑ กรัม/กิโลกรัม น้ำผลิตประโยชน์ขึ้น, และวัสดุดังกล่าว เมื่อใช้สารอาหารเพลิงและปลอดภัยของ
น้ำผลิตมัลทาชื้อขึ้นปริมาณ ๔ กรัม/กิโลกรัม หนู (mice) สามารถรับความปัสสาวะได้ร้อยละ ๖๓.๑ ซึ่งเป็นผลที่ใกล้เคียงกับการ
ใช้ยาอิมมูนโค้ดเพลิง ๑.๕ มิลลิกรัม/กิโลกรัม น้ำผลิตประโยชน์ขึ้น, สำหรับการตรวจสอบความเป็นพิษในระดับเซลล์โดยสัตว์ในนิวคลีอิโอซอล
เซลล์มีสัดส่วนของสารพันท์ของสัตว์คราม ในการตรวจสอบนั้นปริมาณ CD4/CD8 ของเซลล์มีผลต่อ CD4, ต่อ BCTY ๕๐๐
ไม่ได้กำหนด/มิลลิกรัม ทำให้มีการลดเซลล์มีผลต่อเซลล์กระเจิงร้อยละ ๘๔.๐๓ และดัชนีปริมาณเซลล์เป็นร้อยละ ๑๑๐.๒๕ เมื่อเทียบกับ
ค่าควบคุมที่ร้อยละ ๑๐๐ สำหรับการวัด CD4 และ CD8 ได้ร้อยละ ๑๐๙.๗๓ และ ๕๘.๐๐ ตามลำดับ

คำอธิบาย: น้ำผลิตมัลทาชื้อขึ้น, ผลดอกระเบียบวิภูมิเกี่ยวกัน, สารกัดที่ไม่มีพิษ, ไม่มีนิวคลีอิโอเซลล์ของรูปรูป, ภาวะพิทยาศาสตร์