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ANTIPYRETIC ACTIVITY OF *Clitoria ternatea var. pleniflora* (BUTTERFLY PEA) BLUE FLOWER EXTRACT ON YEAST-INDUCED FEVER ON *Mus musculus* (ALBINO MICE)

Lynette B. Gerra, Joanna Rose B. Saligan, Katelyn G. Cericos, Lara M. Garcia, Kirsten Charmaine C. Kanen, Enrico B. Larrazabal, John Michael L. Lumapas, Harvi Marie M. Nisnisan, Mariko P. Sugiyama

College of Medicine
Cebu Doctors' University

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Corresponding Author: Lynette B. Gerra, Joanna Rose B. Saligan, Katelyn G. Cericos, Lara M. Garcia, Kirsten Charmaine C. Kanen, Enrico B. Larrazabal, John Michael L. Lumapas, Harvi Marie M. Nisnisan, Mariko P. Sugiyama, College of Medicine, Cebu Doctors' University, Mandaue City, Cebu, Philippines

Abstract: Fever is the elevation of body temperature ≥ 38ºC. Overdose with over-the-counter drugs used to treat fever, such as paracetamol, can cause side effects like thrombocytopenia and liver failure. This study demonstrates the antipyretic activity of *Clitoria ternatea var. pleniflora* (Butterfly Pea) blue flower ethanolic extract on yeast-induced fever on albino mice. Blue flowers of *Clitoria ternatea var. pleniflora* were collected and extracted using soxhlation. Thirty-two albino mice were used and divided into four treatment groups: a positive control group treated with paracetamol (120mg/5mL) and three experimental groups treated with varying concentrations of the blue flower extract (200 mg/kg, 300 mg/kg, and 400 mg/kg). The rectal temperatures were recorded at 30 minutes, 1, 2, 3, and 4 hours after administration of the different concentrations of the blue flower extract. The antipyretic activity of the blue flower ethanolic extract was determined by comparing the rectal temperatures of the mice in the experimental groups to the rectal temperatures of the mice in the positive control group at each time interval. Result of one-way ANOVA showed that the mean post-treatment rectal temperatures of the albino mice treated with paracetamol, 400 mg/kg, 300 mg/kg, and 200 mg/kg of blue flower ethanolic extracts were not significantly different after 30 minutes ($F = 0.009$, $p = 0.999$), 1 hour ($F = 0.812$, $p = 0.498$), 2 hours ($F = 0.237$, $p = 0.870$), 3 hours ($F = 0.920$, $p = 0.444$), and 4 hours ($F = 1.409$, $p = 0.261$), hence, *Clitoria ternatea var. pleniflora* blue flower ethanolic extract exhibited antipyretic activity.

Keywords: Antipyretic, butterfly pea flower, *Clitoria ternatea var. pleniflora* ethanolic extract

I. INTRODUCTION

Clitoria ternatea, also known as butterfly pea, is a perennial herbaceous plant from the Fabaceae family. The plant grows best with ample moisture and temperatures reaching ≥27°C and produces pentamerous zygomorphic pea-shaped flowers with a tubular calyx made up of five petals (Oguis et al., 2019). The plant has a pantropic distribution. Among the three varieties, the dried petals of the blue *var. pleniflora* are typically used as an herbal tea concoction and the blue color extracted from the flowers is used in the pharmaceutical industry. Phytochemically, the flower contains phlobatannins, flavonoids, and terpenoids (Chusak et al., 2018; Gaytos & Lumagbas, 2019).

According to Aronoff and Neilson (2001), antipyretic drugs reduce fever by inhibiting the enzyme cyclooxygenase (COX), reducing the production of Prostaglandin E2 (PGE2) within the hypothalamus. Examples of these drugs are paracetamol, aspirin, ibuprofen, and naproxen. The two most widely used antipyretics are paracetamol and ibuprofen (Terrie, 2012). Comparing the two, paracetamol is cheaper, has fewer interactions with other drugs, and is safer during pregnancy (Sison, 2022). In the Philippines, paracetamol is an over-the-counter drug that is frequently advertised in the media which is why people usually take it for pain or fever (Bandiola, 2019). The evident mass preference for paracetamol made the drug the positive control of choice for this paper. However, the drug can cause thrombocytopenia, anaphylaxis, bronchospasm, and liver failure. It can also increase the risk of bleeding when used with coumarins (Rotundo & Pyrsopoulos, 2020). Because of these adverse reactions, there is a need to explore the possibility of using less toxic herbal alternatives. One such plant to consider is *Clitoria ternatea var. pleniflora*.

Clitoria ternatea var. pleniflora has been widely used in traditional medicine, specifically to help alleviate various symptoms, such as fever, inflammation, and pain, and diseases such as diabetes (Chusak et al., 2018). There have been studies on the plant's efficacy as an antioxidant, anticancer, antidiabetic, antipyretic, utilizing its roots, stems, and leaves (Al -Snafi, 2016).

The antipyretic activity of the roots was attributed to the presence of flavonoids, which are also present in the flower (Kumar et al., 2017). The flavonoids produce an antipyretic effect that is expressed through the suppression of Tumor Necrosis Factor-α (TNFα), PGE2, and COX production, which are compounds responsible for fever (Abbasi et al., 2018; Shukla et al., 2010). This study aims to determine the antipyretic activity of *Clitoria ternatea var. pleniflora* blue flower extract on yeast-induced fever in albino mice.

II. METHODOLOGY

The study utilized a single-blind randomized controlled trial design, to determine the antipyretic activity of *Clitoria ternatea var. pleniflora* on yeast-induced fever in albino mice.

The experiment was performed at the Experimental Research Laboratory and the Animal Research Laboratory of Cebu Doctors' University, located at 1 Dr. P. V. Larrazabal Avenue, North Reclamation, Mandaue City, Cebu, Philippines, after undergoing ethical review and approval by the Cebu Doctors' University Institutional Animal Care and Use Committee (IACUC).

A total of thirty-two healthy albino mice of either sex, each weighing between 22 to 23 grams, with a baseline rectal temperature between 34°C to 38°C, without any illness, and not part of previous experiments, were used in this study. Excluded from the experiment were albino mice that had any visible physical

deformities, those that were pregnant, those that had a temperature of 39°C or more prior to the induction of fever, and those that did not have an increase in rectal temperature of at least 0.5°C after induction of fever. A licensed veterinarian supervised the handling, determination of which albino mice fulfilled the inclusion and exclusion criteria, as well as the animal euthanasia. The animal subjects were assigned to one positive control group and to the three experimental groups using a random number generator resulting in eight randomly assigned animal subjects per group.

Baseline rectal temperatures were determined using a digital rectal thermometer with a measurement range of -50°C to 300°C and coated with a water-based lubricant. The reset or calibration button was pressed and held down after obtaining each rectal temperature. A stopwatch was utilized to determine the time intervals for data recording (Roy et al., 2019).

Fever was induced by administering 20% Brewer's yeast solution subcutaneously at 10 mL/kg body weight of the mouse (Dangarembizi et al., 2017).

The blue flowers of *Clitoria ternatea var.pleniflora* were purchased from Tisa, Cebu City, and were identified and certified by the Department of Agriculture Regional Field Unit VII in Maguikay, Mandaue City, Cebu, Philippines. The extract was prepared by washing the flowers with distilled water. These were then air dried for one week at room temperature away from direct sunlight. The sample was considered dried when the results of two consecutive weight determinations at one day intervals do not differ by more than 0.5 mg. The dried flowers were then ground into coarse powder using a blender and were then stored at room temperature in an airtight jar until needed.

About 180 grams of the powdered flower of *Clitoria ternatea var. pleniflora* were subjected to extraction with 650 mL of 95% ethanol in a Soxhlet apparatus for 8 hours at 60ºC, and the filtrate concentrated in a rotary evaporator at a temperature of 60°C. Afterward, the filtrate was put in a 50°C water bath to remove the remaining solvent until the solvent odor was no longer evident. The extract obtained was a dark green to black liquid and was stored at room temperature in an airtight amber glass container.

A portion of the flower ethanolic extract was subjected to phytochemical screening to determine the presence of phytochemical constituents.

The density of the flower ethanolic extract was determined to be 1 g/mL. The extract was then diluted with enough distilled water to produce a 1 g/20 mL solution. This served as the stock solution. The doses that were administered to the experimental groups were 200 mg/kg, 300 mg/kg, and 400 mg/kg.

Paracetamol suspension (120 mg/5 ml) was used for the positive control of the study. The recommended dose of Paracetamol is 1 g/70 kg, which has an equivalent mouse dose of 2.6 mg/20 g.

All mice included in the experiment were subjected to fasting for 23 hours. This was done to avoid any chemical interactions between the food and the solutions and for faster absorption of the solution being administered. Water was allowed ad libitum during the experiment period (Roy et al., 2019).

Nineteen hours after fever was induced, the mice were administered their respective treatments via oral gavage using a 20-gauge plastic feeding tube about 1.5-2.0 inches long with a smooth, rounded tip. Paracetamol at a dose of 2.6 mg/20 g was administered to the albino mice of Group I (positive control group). Two hundred milligrams per kilogram, 300 mg/kg, and 400 mg/kg of the blue flower ethanolic extract were administered to Groups II, III, and IV (experimental groups), respectively. Rectal temperatures were taken and recorded at 30

minutes, 1, 2, 3, and 4 hours after administration of the treatments (Parimaladevi et al.,2003). The researchers who took the rectal temperatures did not know what treatment was given to a specific mouse. The antipyretic activity of the blue flower ethanolic extract was determined by comparing the rectal temperatures of the different treatment groups to the temperatures of the positive control group at each time interval after administration of the extract.

One-way Analysis of Variance (ANOVA) was used to determine whether there was a significant difference in the rectal temperatures taken at different time intervals after the administration of test solutions among the positive control and experimental control groups. IBM SPSS version 22 was used for the data processing and analysis.

III. RESULTS AND DISCUSSION

The results of phytochemical screening showed that the flower ethanolic extract was positive for alkaloids, flavonoids, tannins, saponins, and terpenoids. The tables below presented the rectal temperatures of the albino mice pre- and post-induction of fever, which were the baseline and yeast-induced fever rectal temperatures.

Table 1. Pre-treatment baseline and rectal temperatures of the different groups after Brewer's Yeast administration

Treatment Groups	Baseline Temperatures in ℃		*RT in °C 19 hours after Brewer's Yeast Injection	
	℃	SEM**	℃	SEM**
Paracetamol (2.6 mg/20 mg)	35.86	0.41	36.95	0.39
400 mg/kg ethanolic blue flower extract	36.40	0.25	37.16	0.25
300 mg/kg ethanolic blue flower extract	36.20	0.27	37.29	0.21
200 mg/kg ethanolic blue flower extract	36.54	0.41	37.41	0.37

*RT= Rectal Temperature

**SEM= Standard Error of Mean; it signifies how far the reported mean is from the possible means that can be computed. The smaller the value, the more precise and reliable the reported mean.

Table 1 shows the mean baseline temperatures of the mice treated with Paracetamol, 400 mg/kg, 300 mg/kg, and 200 mg/kg of blue flower ethanolic extracts. All the baseline temperatures were within the normal range of 34-38°C. Their temperatures after the induction of fever increased, showing the effect of Brewer's yeast in inducing fever by increasing the synthesis and release of different cytokines such as interleukins, tumor necrosis factor, and prostaglandins (Dangarembizi et al., 2018). The yeast also binds to the lipopolysaccharide-binding protein, leading to the synthesis and release of PGE2, which then binds to prostaglandin E receptor 3 in the median preoptic nucleus of the hypothalamus, resulting in an increase in body temperature (Dangarembizi et al., 2018). The mean rectal temperatures of the different groups were recorded, and their SEM calculator as well.

Table 2. Post-treatment Rectal Temperatures of Albino Mice of the Groups at Different Time Intervals

Treatment groups	Rectal Temperatures (in °C) After									
	0.5H		1H		2H		3H		4H	
	°C	SEM	°C	SEM	°C	SEM	°C	SEM	°C	SEM
Paracetamol (2.6 mg/20 mg)	36.31	0.25	36.03	0.37	35.73	0.18	36.29	0.27	36.70	0.30
400 mg/kg ethanolic blue flower extract	36.39	0.49	35.76	0.44	35.99	0.34	36.19	0.32	36.75	0.32
300 mg/kg ethanolic blue flower extract	36.34	0.36	36.55	0.33	36.76	0.28	35.68	0.39	36.10	0.19
200 mg/kg ethanolic blue flower extract	36.38	0.29	36.13	0.30	35.69	0.28	35.64	0.41	36.24	0.27

Table 2 presents the mean rectal temperature 30 minutes after treatment administration. The lowest mean temperature at this time interval was that of Paracetamol at 36.31°C and this coincided with the onset of action 0.5-2 hours after administration. Mice treated with 400 mg/kg blue flower ethanolic extract had the highest mean temperature of 36.39°C at 39 minutes, whereas those treated with 300 mg/kg blue flower ethanolic extract exhibited a mean rectal temperature of 36.34°C, and those treated with 200 mg/kg blue flower ethanolic extract exhibited a mean rectal temperature of 36.38°C. It was noted that all the temperatures of the mice at 30 minutes had decreased as compared to their yeast-induced fever temperatures, possibly attributable to the antipyretic activity of *Clitoria ternatea var. pleniflora* blue flower ethanolic extract. The mean rectal temperature after 1 hour of the group treated with Paracetamol was 36.03°C, which had further decreased as compared to the rectal temperature taken 30 minutes after administration of treatment. Mice treated with 400 mg/kg blue flower ethanolic extract had the lowest mean temperature of 35.76°C. Those treated with 300 mg/kg blue flower ethanolic extract had the highest mean rectal temperature of 36.55°C, and those treated with 200 mg/kg blue flower ethanolic extract had a mean rectal temperature of 36.13°C. Those treated with 300 mg/kg blue flower ethanolic extract had the highest mean rectal temperature, which may likely be due to the lower concentration of the extract as compared to the 400 mg/kg group, leading to a slower peak antipyretic activity.

The mean rectal temperatures taken 2 hours after administration of the different treatments were 35.73°C, 35.99°C, 36.76°C, and 35.69°C, respectively, for the Paracetamol group, the 400 mg/kg dose, 300 mg/kg dose, and 200 mg/kg dose. The mean rectal temperature of the group treated with Paracetamol after 2 hours was 35.73°C which is even lower than that of the temperature 1 hour after administering the treatment. Mice treated with 200 mg/kg blue flower ethanolic extract had the lowest mean rectal temperature of 35.69°C. It is noted that there is a further decrease in temperature at this time period in comparison with the temperature 1 hour prior. Those treated with 300 mg/kg blue flower ethanolic extract had the highest mean rectal temperature of 36.76°C. Lastly, those treated with 400 mg/kg blue flower ethanolic extract exhibited a mean rectal temperature of 35.99°C.

Table 3. One-way ANOVA for Post-treatment Rectal Temperatures of the Treatment Groups

Time	F-value	*p* value*	Conclusion
0.5H	0.009	0.999	Not significant
1H	0.812	0.498	Not significant
2H	0.237	0.870	Not significant
3H	0.920	0.444	Not significant
4H	1.409	0.261	Not significant

*p-Values are greater than 0.05 (*p-value<0.05*

Table 3 shows the comparison of post-treatment rectal temperatures of the different groups analyzed through one-way ANOVA. Results showed that post-treatment rectal temperatures at 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours have no significant difference and contain the same p-values greater than the 0.05 level of significance (*p<.05*). This means that the rectal temperatures of the positive control group and of the different *Clitoria ternatea var. pleniflora* blue flower ethanolic extracts had no difference, thereby, proving that all doses of *Clitoria ternatea var. pleniflora* blue flower ethanolic extract had comparable antipyretic activity to Paracetamol.

IV. CONCLUSION

The *Clitoria ternatea var. pleniflora* blue flower ethanolic extract (200 mg/kg, 300 mg/kg, and 400 mg/kg) had an antipyretic effect comparable to that of Paracetamol. Results showed that the post-treatment rectal temperatures of the albino mice treated with Paracetamol, 400 mg/kg, 300mg/kg, and 200 mg/kg blue flower ethanolic extracts did not show any significant difference even after only 30 minutes.

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