Dama International Journal of Researchers (DIJR), ISSN: 2343-6743, ISI Impact Factor: 0.878
Vol 2, Issue 2, February, 2017, Pages 9 - 15 Available @ www.damaacademia.com

The Antipyretic Effects Test Of Ethanol Extracts Of Green Chiretta Herbs (*Andrographis Paniculata Nees*) on Male Mice (*Mus musculus*)

Sainal Edi Kamal[1], Zulfiah[2], Rina Asrina[3], Muh. Farid Hasyim[4], Agus Dwi Jayanti[5]
[1,2,3,4] *Sandi Karsa Pharmaceutical Academy of Makassar, Indonesia*
[5]*Yamasi Pharmaceutical Academy of Makassar, Indonesia*

Abstract
The antipyretic effects test of ethanol extracts of green chiretta herbs on male mice has been conducted with the purpose of obtaining scientific data as traditional medicine. This test used 18 male mice that were divided into 6 groups, 4 groups were given extract orally as much as 1ml/30grams body weight of male mice, with a concentration of 1% w/v, 2% w/v, 4% w/v, and 8% w/v as well as the comparison of paracetamol suspension 0.02% w/v and Na.CMC control 1% w/v. The male mice were fasted before the treatment, and then 10% peptone solution is injected intraperitoneally. The temperature is measured again after the administration of the green chiretta ethanol extract paracetamol suspension, and Na.CMC 1% w/v. The research results showed that all of the concentrations of green chiretta herbs ethanol extract can lower the body temperature of male mice. The drop in temperature for the green chiretta herbs ethanol extract concentration of 8% w/v shows the effect of temperature decrease is not significantly different from paracetamol suspension of 0.02% w/v at the level of 5% ($\alpha = 0.05$).

Keywords: *Green Chiretta Herbs, Antipyretic, Male Mice*

I. INTRODUCTION

Antipyretics are drugs or substances that can reduce body temperature or reduce fever. Fever is usually caused by the body's exposure to infectious microorganisms (viruses, bacteria, parasites). Fever may also be caused by non-infectious factors such as immune complexes, or other inflammations. When a virus or bacteria enters the body, the various types of white blood cells or leucocytes release fever-causing substances (endogenous pyrogen) which in turn triggers the production of prostaglandin E2 in the anterior hypothalamus which in turn increases the value of the threshold temperature and there was fever. During fever, the hypothalamus carefully controls the temperature rise so that the body temperature rarely exceeds 41^{o}C (Dinda, 2008). Today the research and development of medicinal plants both nationally and abroad is growing rapidly. Research has mainly grown in terms of its pharmacological and phytochemical based on indication of herbs that have been used by some people with efficacy proven empirically. The research results, of course, further solidify the users of medicinal plants of the efficacy as well as its usefulness. (Dalimartha, 2000) In Indonesia, traditional medicine has been going on since a long time ago, and folk remedies have been used widely for generations. Generally, folk remedies are used for the prevention, treatment, and increasing endurance. In the national health system, traditional medicines were used alongside modern medicine and other health facilities. (Wijayakusuma, 2000)

Traditional medicines are traditionally processed, hereditary drugs based on the recipes of ancestors, customs, beliefs, or local customs, and traditional knowledge. According to the present research, traditional medicines are beneficial to health, and now intensified its use because it is not causing side effects and more accessible to the public, in both price and availability. (Hasan, 2006). The use of plants as medicine has been widely recognized both in developing and developed countries. This was strengthened by the thought back to nature as well as the prolonged crisis which caused a decline in society's purchasing power. In Asia and Africa, 70-80% of the population still depends on traditional medicine as a primary treatment. The widespread use of traditional medicine caused by the public trust that traditional medicines are made from natural ingredients, are safer and does not cause any side effects. (WHO, 2009). The efficacy of green chiretta (*Andrographis paniculata* Nees) as one of the ingredients of traditional medicine has been widely recognized since the past, by both Indonesian and other nations of the world. The popularity of green chiretta in traditional medicine were without doubt because it is proven efficacious and able to cure a variety of illnesses, from mild to severe. The numerous efficacy of green chiretta cannot be separated from its active substances that are so complete. Among the chemical content contained in green chiretta is the andrographolide which is the bitter compound that can reduce fever (Prapanza et al., 2003). Based on this background, the formulation of the problem is whether the green chiretta herb's ethanol extracts can demonstrate the antipyretic effect on male mice? and at what concentrations the ethanol extracts of green chiretta herb can show antipyretic effect? The purpose of this study is to determine the effectiveness of green chiretta plant as a natural ingredient that has the antipyretic efficacy toward male mice at various concentrations.

II. RESEARCH METHOD

A. Tools and Materials

The tools used in this research include: autoclave, aluminum foil, rod stirrer, funnel glass, 500 ml Erlenmeyer (Pyrex®), 100 ml measuring cup (Pyrex®), 500 ml beaker (Pyrex®), oral needle, mortar and pestle, mouth blocks, pH meter, Rotavapor, syringe (Terumo), stopwatch, rectal thermometer (MT.B122), analytical balance, animal weighing scale (O'hauss®). The Materials used in this research are: distilled water, alcohol 70%, water for injection, male mice (Mus musculus), sodium carboxymethyl cellulose (Na.CMC) 1% w/v, peptone 10% w/v, samples of green chiretta herbs (*Andrographis paniculata* Ness), paracetamol tablets.

B. Time and Place of Research

The research was conducted in June to August 2016, was conducted in the Biopharmaceutical Laboratory of Sandi Karsa Pharmaceutical Academy, Makassar.

C. Population and Sample

1. Population

a. Green Chiretta
   Green Chiretta plant grows wild in the streets, abandoned fields, and open spaces exposed to the sun.
b. Mice
   The research's population was selected adult, able bodied, local strain of mice weighing 20-30 grams.

2. Sample

a. Green Chiretta Herbs (*Andrographis paniculata* Ness)
   Samples were taken in Makassar area, precisely on the lawn of the residential area of Bontolempangan residences.
b. Mice (Mus musculus)
   The determination of the amount of samples in this research is based on the Federer formula, which is (t - 1) (n – 1) > 15. Whereas t is the treatment group and n is the amount of samples.

D. Data Collection Technique

1. Retrieving and processing samples

a. Retrieving samples
   Samples of green chiretta herbs (*Andrographis paniculata* Ness) were collected in Makassar area.
b. Processing samples
   Samples of green chiretta herbs were collected, fresh plants were selected, then dried by means of aeration in a shady place and protected from direct sunlight, then cut into small pieces in accordance with fine degrees of 4/18 equivalent to 0.25 cm / 0.06 cm, then extracted with maceration method.

2. Preparation of ethanol extract of green chiretta herbs with maceration method

Samples of green chiretta herbs that has turned into powder weighed as much as 500 grams then inserted into the maceration vessel. 1000 ml of 70% ethanol solvent was added into the vessel. The vessel was closed using aluminum foil and stored in a placed that is protected from direct sunlight. Extracted for 5 days while occasionally stirred, maceration filtered and then collected. The waste is macerated again 3 times, each maceration using 1000 ml of 70% ethanol until the last extract becomes clear. The liquid ethanol extract is evaporated using rotavator until thick extract weighing as much as 20 mg is obtained.

3. *Preparation of Colloidal solution Na.CMC 1% w/v*

1 gram of Na.CMC powder is weighed, the put into as much as 7 ml hot distilled water (at 70 oC) little by little while stirring at high speed using electric mixer till a homogenous colloidal solution is formed. Then distilled water is added till the total volume reach 100 ml.

4. Preparation of peptone solution 10% w/v

A total of 10 grams of peptone dissolved into 80 ml of distilled water which were then checked at pH 6.8 and the volume added to 100 ml, then sterilized in an autoclave at 121 oC for 20 minutes.

5. Preparation of paracetamol suspension

A total of 20 paracetamol tablets were weighed and then the average weight was calculated. Crushed in a mortar and the paracetamol powder equivalent to 0.02 w/v was weighed, then suspended with 50 ml Na.CMC Colloidal solution of 1% w/v in a mortar until it became homogenous, then the volume is added with Na.CMC Colloidal solution of 1% w/v till it reach 100 ml.

6. Selection and Preparation of Test Animal

a. Selection of Test Animal
   Test Animal used were healthy, adult male mice (Mus musculus) weighed 20 - 30 grams and adapted for 2 weeks.
b. Preparation of Test Animals
   A total of 18 Adult male mice were used which were divided into six groups and each group consisted of 3 mice. Group I was treated with 1% Na.CMC solution as the control, group II (1% w/v), III (2% w/v), IV (4% w/v) and V (8% w/v) each were given green chiretta leaf ethanol extract as the test group, and group VI (0.02% w/v) were given paracetamol suspension as a comparison.

7. Treatment of Test Animals

a. Treatment of the mice prior to fever
   Before the mice were injected with peptone, they were first fasted for 12 hours and given only water to drink. All groups of mice have their rectal temperature measured. The measurement results were recorded as the initial rectal temperature.
b. Injecting 10% w/v peptone solution to mice intraperitoneally
   Mice that had their initial rectal temperature measured injected with 10% w/v sterile peptone solution intraperitoneally (i.p). Observation of peak fever were carried out by re-measuring the rectal temperature of mice every 30 minutes until the body temperature of the mice is relatively constant.
c. Treatment of mice that have been induced with fever by means of administrating green chiretta herbs ethanol extract
   After observation of the peak temperature of fever, the mice in group II, III, IV and V were given green chiretta herbs ethanol extract with a dose of 1 ml / 30 grams of the body weight of male mice orally with a concentration of 1% w/v, 2% w/v, 4% w/v, and 8% w/v. Observations of the fever temperature decrease was done by re-measuring the rectal temperature of mice every 30 minutes until the body temperature of mice is relatively constant.
d. Administering paracetamol suspension
   Group VI, were given paracetamol suspension of 0.02% w/v with a dose of 1 ml / 30 grams body weight of male mice orally as a comparison.
e. Administering 1% w/v Na.CMC solution as control
   To mice in group I, they were treated with Na.CMC solution with a dose of 1 ml / 30 grams body weight of mice orally as the control.

8. Observation and Data Collection

Each group that was given treatment, were placed in a cage and then observed by measuring their data in accordance with the observation

E. Analyzing Technique

This research uses experimental method namely the Post Test Only Controlled Group Design, which is a type of research that only make observations on the control and treatment groups after being given an action. The data obtained from the research result will be analyzed statistically using the Variance Analysis. Then it will be discussed and conclusions will be drawn.

III. RESULTS

From the normal temperature measurement results, fever temperature and after measurements on the male mice after administering the ethanol extract of green chiretta herbs:

1. The measurement results of the initial rectal temperature, fever temperature and after treatment can be seen in Table I
2. The average increase and decrease of the measurement on the normal temperature, fever temperature, and the temperature after the treatment in male mice using the ethanol extract of green chiretta herbs can be seen in Table II.
3. The percentage of temperature drop after administering Na.CMC, ethanol extract of green chiretta herbs and paracetamol suspension 0.02% w/v on male mice can be seen in Table III.

Table 1. Measurement results of normal temperature, fever temperature and after-treatment temperature on male mice by using the ethanol extract of green chiretta herbs *(Andrographis paniculata (Ness)*.

Treatment	N	Normal temperature (°C)	The temperature of the fever (°C)	Temperature after treatment (°C)
Na.CMC 1% b/v (control)	1 2 3	32.1 32.5 31.7	39.3 35.7 34.8	35.2 35.5 34.7
Average		32.1	36.6	35.1
Extract 1 % b/v	1 2 3	32.3 31.9 32.2	35.6 35.8 35.7	35.3 35.4 35.1
Average		32.1	35.7	35.2
Extract 2 % b/v	1 2 3	31.8 31.7 32.4	35.7 35.5 35.7	34.7 34.4 34.4
Average		31.9	35.6	34.5
Extract 4 % b/v	1 2 3	32.3 32.4 32.3	35.7 35.6 35.5	34.3 34.2 34.0
Average		32.3	35.6	34.1
Extract 8 % b/v	1 2 3	31.7 32.2 31.9	35.8 35.7 35.9	34.1 33.8 33.9
Average		31.9	35.8	33.9
Parasetamol (comparison) 0.02 % b/v	1 2 3	32.2 31.1 32.1	35.9 35.6 35.7	33.7 33.7 33.8
Average		31.8	35.7	33.7

Table 2. Average increase and decrease of temperature in the measurement of normal temperature. fever temperature. and after-treatment temperature on male mice using the ethanol extract of green chiretta herbs *(andrographis paniculata (Ness)*

Treatment	N	Normal temperature (°C)	The temperature of the fever (°C)	Temperature rise (°C)	Temperature after treatment (°C)	Decreased temperature (°C)	% Decrease
Na.CMC 1% b/v (control)	1 2 3	32.1 32.5 31.7	39.3 35.7 34.8	3.2 3.2 3.1	35.2 35.5 34.7	0.1 0.2 0.1	3.13 6.25 3.22
Average		32.1	36.6	3.1	35.1	0.1	4.2
Extract 1% b/v	1 2 3	32.3 31.9 32.2	35.6 35.8 35.7	3.3 3.9 3.5	35.3 35.4 35.1	0.3 0.4 0.6	9.09 10.25 17.14
Average		32.13	35.7	3.57	35.27	0.43	12.16
Extract 2% b/v	1 2 3	31.8 31.7 32.4	35.7 35.5 35.7	3.9 3.8 3.3	34.7 34.4 34.4	1.0 0.6 0.8	25.64 15.79 24.24

Average		31.97	35.63	3.67	34.5	0.8	21.89
Extract 4% b/v	1 2 3	32.3 32.4 32.3	35.7 35.6 35.5	3.4 3.2 3.2	34.3 34.2 34.0	1.4 1.4 1.5	41.17 43.75 46.87
Average		32.3	35.6	3.27	34.17	1.43	43.93
Extract 8 b/v	1 2 3	31.7 32.2 31.9	35.8 35.7 35.9	4.1 3.5 4.0	34.1 33.8 33.9	1.7 1.9 2.0	41.46 54.28 50.00
		31.93	35.8	3.87	33.93	1.87	48.58
Parace-tamol (compari-son) 0.02% b/v	1 2 3	32.2 31.1 32.1	35.9 35.6 35.7	3.7 3.5 3.6	33.7 33.7 33.8	2.2 1.9 1.9	59.46 54.28 52.78
Average		31.8	35.73	3.6	3373	2	55.67

Table 3. Percentage of temperature decrease after administering Na.CMC. ethanol extract of green chiretta herbs *(Andrographis paniculata.Ness.)* and paracetamol suspension on male mice (Mus musculus)

N	Na.CMC 1% b/v As control	Ethanol extract of green chiretta				Paracetamol 0.02% b/v
		1%	2%	4%	8%	
1 2 3	3.13 6.25 3.22	9.09 10.25 17.14	25.64 15.79 24.24	41.17 43.75 46.87	41.46 54.28 50.00	59.46 54.28 52.78
∑	12.6	36.48	65.67	131.79	145.74	166.52
X	4.2	12.16	21.89	43.93	48.58	55.51

Glossary:
N : Test animal (Replication)
X : The average amount of temperature decrease
∑ : The total amount of temperature decrease
Appendix 1 : Statistical data processing using Completely Randomized Design (CRD) and Newman Keuls Advanced Test. The processed data is taken from Table 3

IV. DISCUSSIONS

In the beginning of this research we were using sample from fresh Green Chiretta leaves. used in male mice due to it's stable hormonal system compared to female mice that owns estrus cycle. This cycle could significantly affect the research result. Before the treatment began the male mice adapted so it would turn docile and less stressful when the treatment began. On the next phase male mice would need to fast to nullify the effect of food to the experiment material. In this experiment we were using paracetamol as a comparison because it consumed by public in general and thus have the mildest side-effect compared to another antipretik medicine. Paracetamol was made in the form of suspension, specifically in the form of Na.CMC 1% b/v, Na.CMC used as control agent. Control agent used to observe experiment material potency to reduce the fever degree. In order to make the male mice get a fever we were using pepton. Fever inflicted with injection of 10% steril 0.6 ml pepton liquid with intraperitonial ways. So far pepton is the easiest, safest and easier to find that is the reason we choose it. Fever degree measured till it reaches the optimum degree compared to normal body heat. In this experiment we were using rectal measurement to measure the body heat until it reach normal degree. We were using rectal because rectal is the only part of the body that didn't change it's temperature while affected by enviroment.

Rectal temperature is the most stable temperature compared to another part of the body because it didn't affected by enviroment temperature. If we measure the temperature in the mouth of axilla it would cause difference due to the breathing frequency. In this research we were using pepton as fever inflicting substance to the male mice because pepton contain pirogen substance which will cause fever better when injected to the male mice compared

to yeast vaccine or sterile milk. This active substance work in such mechanism called andrographolide served as analgetic and antipiretic in such way it raise up the betaendorfin level in plasma. this mechanism work to reduce fever. Betaendorfin is a neurotransmitter that contain analgetic effect (reducing pain) and antipiretic (reducing fever).

Based on the research where Green Chiretta Herbal Extract with concentration of 1%, 2%, 4% and 8% with Paracetamol suspension as comparison and Na.CMC as control agent that injected to male mice that induced with pepton substance shown certain variable of fever degree reduction. When injected with concentration of 1%, 2%, 4% and 8% each one reduced on average of (12.16%), (21.89%), (43.93%), (48.59) meanwhile the one injected with paracetamol 0.02% b/v and Na.CMC 1% b/v, shown temperature reduction of (55.51%) and (4.2%). Based on the data that we provide above which etanol extract Green Chiretta with concentration of 1%, 2%, 4% and 8% shown less temperature drop compared to paracetamol. While if we look at histogram data we could find that temperature drop precentage of Na.CMC is 4.2%. For sambiloto extract with concentration of 1%, 2%, 4% and 8% and paracetamol suspension 0.02% is 12.16%, 21.89%, 43.59% and 55.51%. Data shown that etanol herbal Green Chiretta with 8% concentration have the largest contribution in reducing the fever temperature compared to 1%, 2%, 4% concentration. This caused due to the large dose given so in that level of concentration the active substance is high enough to decrease the temperature.

The result of statistical analysis obtained from ANOVA table shows that F count (40.64) is greater than F table 5% (3.48) and 1% (5.99). This shows that there is a treatment effect (concentration) towards fever temperature decrease in male mice. In the advanced tests using the Newman Keuls test significant results were obtained in a concentration of 1%, 2%, 4% which means that at these concentrations provide significantly different results. Similarly, at concentration of 8% compared with paracetamol suspension as a comparison the result obtained were non-significant. This shows that at a concentration of 8%. the ethanol extract of green chiretta herbs effects were not significantly different with paracetamol suspension 0.02% w/v towards fever temperature decrease.

V. CONCLUSIONS

Based on research results. discussion and analysis of the data obtained. it can be concluded as follows:

1. This research results showed that all concentrations of the ethanol extract of green chiretta herbs can lower the body temperature of male mice
2. Ethanol extract of green chiretta herbs at a concentration of 8% w/v statistically were not giving a significantly different result with the administration of paracetamol suspension 0.02% w/v at the trust level of 5% ($\alpha = 0.05$)

VI. RECOMMENDATIONS

It is suggested for subsequent researchers to investigate other effects of the ethanol extract of green chiretta herbs to add to scientific data. especially in the pharmaceutical field.

REFERENCES

1. Dalimartha. S. 1999. Atlas Tumbuhan Obat Indonesia Jilid I. Jakarta: TrubusAgriwidya
2. Dalimartha. S. 2000. Atlas Tumbuhan Obat Indonesia Jilid II. Jakarta: TrubusAgriwidya
3. Dirjen POM. 1979. Farmakope Indonesia Edisi III.Jakarta: Departemen Kesehatan RI
4. Departemen Kesehatan Republik Indonesia. 1986. Sediaan Galenik. Direktorat Jendral Pengawasan Obat dan Makanan: Jakarta
5. Departemen Kesehatan Republik Indonesia. 1995. Farmakope Indonesia. Edisi IV. Departemen Kesehatan RI. Jakarta
6. Departemen Kesehatan Republik Indonesia. 1978. Farmakope Indonesia. Edisi III. Departemen Kesehatan RI. Jakarta
7. Ganiswarna. S. G. 1995. Farmakologi dan Terapi Edisi IV. Gaya Baru. Jakarta
8. Hariyanto. W. 1991. Mengapa Kita Demam. Arca: Jakarta
9. Ismawan. B. 2008. Herbal Indonesia Berkhasiat. Penerbit PT. Trubus Swadaya: Depok.
10. Kumala. P. 1998. Kamus Saku Kedokteran Dorland. Buku Kedokteran EGC: Jakarta
11. Malole. M.B.M. dan Pramono. C.S.U. 1989. Penggunaan Hewan-hewan Percobaan di laboratorium. Bogor : Departemen Pendidikan danKebudayaan.Direktorat IPB.
12. Prapanza.I.E.P. Dan Marianto. L.A.S.P.. 2003. Khasiat dan manfaat Sambiloto. PT Agro Media Pustaka: Tangerang.
13. Sastrohamidjojo. H. 2005. Kimia Organik Stereokimia. Karbohidrat. Lemak. dan Protein. Gadjah Mada University Press: Yogyakarta
14. Staf Pengajar Departemen Farmakologi Fakultas Kedokteran Universitas Sriwijaya. 2009. Kumpulan Kuliah Farmakologi Edisi 2. Penerbit Buku Kedoteran EGC: Jakarta

15. Tjay. T.H. dan Kirana R. 2007. Obat-obat Penting. Edisi Keenam. Elex Media Komputindo Kelompok Gramedia: Jakarta
16. Van Steen. C.G.J. 1997. Flora. Pradnya Paramita: Jakarta.
17. WHO. 2009. Traditional Medicine. Retrieved Desember 2011. from http://www.who.int/botanical/mediacenter/factsheet/fs134/en/
18. Wibowo. S. 2006. Antipiretik. (on Line). (http://google/seputar-demam.html. acessed 27 Juni 2013)
19. Wijayakusuma. Hembing. 2000. Ensiklopedia Tumbuhan Berkhasiat Obat Indonesia Jilid I. Jakarta: PT. Prestasi Insan Indonesia.