PRACTICAL GUIDEBOOK
PHARMACOLOGY AND TOXICOLOGY

CREATED BY
STAFF AND LABORATORY ASISTANT
DEPARTEMENT OF PHARMACOLOGY PHARMACY

PHARMACOLOGY AND TOXICOLOGY LABORATORY
INTERNATIONAL PROGRAM
FACULTY PHARMACY
UNIVERSITY OF SUMATERA UTARA
2019
PHARMACOLOGY AND TOXICOLOGY LABORATORY  
DEPARTMENT OF PHARMACEUTICAL PHARMACOLOGY  
FACULTY OF PHARMACY  
UNIVERSITAS SUMATERA UTARA  

STUDENT’S BIODATA  

NAME : ________________________________  
NIM : ________________________________  
GROUP : ________________________________  
STUDY PROGRAMME : ________________________________  
FACULTY : ________________________________  
UNIVERSITY : ________________________________

Photo size 3 x 4
STAFF FOR PHARMACOLOGY AND TOXICOLOGY LABORATORY
FACULTY OF PHARMACY USU


Staff Laboratory : Prof. Dr. Urip Harahap, Apt.
Prof. Dr. Rosidah, M.Si., Apt.
Drs. Saiful Bahri, M.S., Apt.
Dr. Poppy Anjelisa Z. Hasibuan, S.Si., M.Si., Apt.
Dr. Edy Suwarso, SU., Apt.
Dr. Khairunnisa, S.Si., M.Pharm., Apt.
Marianne, S.Si., M.Si., Apt.
Hari Ronaldo Tanjung, S.Si., M.Sc., Apt.
Aminah Dalimunthe, S.Si., M.Si., Apt.
Yuandani, S.Farm., M.Si., Apt.
Imam Bagus Sumantri, S.Farm., M.Si., Apt.
Dadang Irfan Husori, S.Si., M.Sc., Apt.

Laborant : Sasniwiati Sari Hasibuan, S.Farm

Assistant Laboratory : Zainul Fuad Nurhadi
Joule De Ceva Magribi
Dhea Nur Fadhillah
Cindi Indriyani
Trya Nur Indah
Kurnia Lavinda Yusfa
Sigit Dui Haryanto
Akbar Pratama
Nurnasuha Zainal Abidin
Ulva Khairani Ritonga
Desy Arianti Panjaitan
Christal Jennifer Grundling
LABORATORY RULES AND REGULATIONS

1. The requirements to enter lab practical are as follows:
   - Students who have taken Pharmacology and Toxicology lecture courses.
   - Students have filled out study plan cards (KRS) to take part in Pharmacology and Toxicology lab practical.
   - Shows a copy of the study plan card (KRS).
   - Colour photo size 3x4: 1 photo
2. Practical starts at 13:30 WIB and students must be on time.
3. During the practical, the students are required to wear lab coats, gloves, masks, name badges and are required to follow the USU Pharmacy Faculty dress code.
4. Every group is responsible for the supply and maintenance of animals used during practical.
5. Every group is responsible for the cleanliness of the table and tools of pacticum and returning the equipment in clean condition.
6. Practical data is declared valid if it has been signed by the assistant on duty.
7. If there is a dangerous situation in the laboratory, the practitioner must immediately report to the lecturer / assistant on duty, and if the lab practice encounters any difficulties, ask for instructions and guidance from the lecturer / assistant on duty.
8. Practitioner that is unable to attend must provide a written letter or a doctor's certificate if sick.
9. Practitioner that skipped on any lab practical are required to carry out the practical activities on other days.
INSTRUCTIONAL OBJECTIVES

A. GENERAL
   After completing this practical, S-1 Regular Pharmacy students will be able to evaluate drug activity using various methods of pharmacological experiments.

B. SPECIFIC
   1. Students can apply good animal handling methods and animal use ethically.
   2. Students can evaluate drug activity based on the Drug Delivery Route Of Administration.
   3. Students can evaluate drug activity based on Biological Variations.
   4. Students can evaluate drug analgesic activity
   5. Students can evaluate drug antipyretic activity.
   6. Students can evaluate the anti-inflammatory activity of drugs.
   7. Students can evaluate diuretic drug activity.
   8. Students can evaluate drug activity on the central nervous system.
   9. Students can evaluate drug activity on the peripheral nervous system.
  10. Students can evaluate drug activity on the digestive system.
  11. Students can evaluate drug activity on the immune system.
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CHAPTER 1. DRUG RESPONSE BASED ON DOSE, ROUTE OF ADMINISTRATIONS, AND BIOLOGICAL VARIATIONS

I. SPECIFIC INSTRUCTIONAL OBJECTIVES
   After completing this experiment, the students will be able to:
   1. Apply a good handling and use of appropriate animal complied with ethics regulation.
   2. Evaluate drug activity based on the dose, route of administration and biological variation.

II. ETHICS IN UTILIZING EXPERIMENTAL ANIMAL
   Innocent experimental animals used in research will suffer from several unwanted conditions, including discomfort, unhappiness, distress, pain, and even sometimes end with death. Due to these facts, the animals sacrificed in any study for the purpose of human being should be treated humanely, well maintained, and customized to have the best care. The researchers who will utilize laboratory animals in medical study should examine the feasibility and the utilization of animals by considering the suffering that will be experienced by the animals and the future benefits to humans.

   In the study process, researchers must prepare and customize a protocol complied with the accepted standards of scientific and ethical health research. Ethic in health research, in general, are listed in the World Medical Association, including: respect (respect the rights and dignity of living beings, freedom of choice and desire, and is responsible for himself, including experimental animals), beneficiary (useful for humans and other creatures, the benefits obtained must be greater than the risks experienced), and justice (fair in making use of animal experiments). Examples of unfair attitude, among other things: repeated injection/dissection of animals to save their number used in a study and using a cheaper drug for euthanasia that causes pain. In health studies that utilize animal, the principle of 3 Rs (Replacement, Reduction and Refinement) should be practiced.

   Replacement is the purpose of utilizing experimental animals that has been carefully calculated, both from previous experience and literature to answer the research question and cannot be replaced by other creatures such as a cell or tissue culture. Replacement is divided into two parts:
   1. Relative replacement (replace animal experiments using organs/tissues of animals from Slaughter houses, animals of a lower order)
2. Absolute replacement (replace animal experiments with cell cultures, tissue, or a computer program).

Reduction is defined as the use of animals in research as minimum number as possible, but still can provide an optimal result. The minimum number of the experimental animals required is usually calculated using the Freederer formula expressed as: 
\[(n-1) (t-1) > 15\]
in which:  is the number of animals required and  is the number of treatment groups. The limitation of the formula is that the fewer the number of the treatment groups, the more the number of animals required, and vice versa. To overcome this problem, an appropriate statistical design is necessary to obtain valid research results.

Refinement is to treat the animals humanely. They should be kept well, avoided from hurting them, and minimizing the painful treatment to guarantee the animal welfare by the end of the study. The basic principles are to avoid the experimental animals from undergoing several conditions. The first is that the experimental animals are free from hunger and thirst by providing access to adequate amount and compositions of food and drinking water to guarantee the animals’ health. Food and drinking water of adequate qualities are proven through food proximate analysis, analysis of drinking water quality and contamination performed periodically. Analysis of animal feed is necessary to get the feed composition using standard methods. The second, the experimental animals should be free from discomfort by providing a clean environment and the most appropriate for the biological life of the selected experimental animals. The following conditions must be highlighted: the light cycle, temperature, humidity, environment, and physical facilities such as the appropriate size of the cage to allow freedom movement of the animals, the habits of animals for group or alone. Additionally, the experimental animals should be free of pain and disease by running health programs, prevention, and monitoring, as well as experimental animals against treatment if necessary. A disease suffered by an experimental animal can be treated as long as it does not interfere with the research being executed. Effort to minimize pain experienced by the experimental animals, mainly in invasive procedure, should be practiced by using analgesia and anesthesia when needed.

III. DRUG DOSE
Dose is the amount of the active ingredient of a certain dosage form that provides specific effects on an illness or symptoms of an illness. A maximum dose is the largest dose that can be given to adults without harm. Ad Infinitum is a condition at which there is no
increase in response (known as maximum response) at the increase of the drug dose. Instead, a minimum dose that can provide a real response is referred to as the threshold dose and the response is called as the response threshold.

For a drug to generate an effect with certain intensity in the population, its dose range is necessary. If plotted frequency distribution of individuals responsiveness (in %) in the dose range (in log dose), dose effects of therapy in 50% of such individuals is called as a therapeutic dose of the median or median effective dose (ED50). Median lethal dose (LD50) is the dose that causes death in 50% of individuals, while the TD50 is toxic dose of 50%. Ideal drugs should provide therapy effects in all patients without causing toxic effects in a patient. Therefore:

\[
\text{Therapeutic index} = \frac{TD1}{ED99} \text{ is more appropriate,}
\]

\[
\text{And for the ideal drug: } \frac{TD1}{ED99} > 1
\]

However, those extreme values can not be determined accurately because they are the part of the curve and the curve is almost flat.

Responses produced by a certain dose of a drug vary from one patient to another. Since not all patients have the same size, body weight, age, and sex, it would be wise to consider how the factors may affect the response and how much medication to be received by a person to produce the required effect of a drug. Recommendations are often used for the treatment with the adult dosage, such as those found in the standard references, based on the assumption that the patient is "a normal" adult. As "normal" (or average) are now said to be 5 feet 9 inches (173 cm) tall and weighs 154 lbs (70 kilograms). However, there are many people who do not fit into this category. In order to obtain an optimal effect of a drug, therefore, body weight, body surface area (BSA), age, sex, genetic factors, clinical and psychological conditions of the patient, tolerance, time of administration, drug interaction, and route of drug administration should be taken into account (Heiserman, 2001).

Drug delivery is a very important factor to consider to achieve the required effect of a drug. Drug delivery affects the onset and duration of drug action. Drug can be delivered intravascularly and extravascularly.

To deliver a drug intravenously, the needle used should be sharp and with appropriate size. Appropriate needle size and the maximum volume for a variety of administration route can be seen in Table 1.
Table 1. Methods of administration of drugs to animals and the size of the syringe

<table>
<thead>
<tr>
<th>Animals</th>
<th>Syringe</th>
<th>i.v.</th>
<th>i.p.</th>
<th>s.c.</th>
<th>i.m.</th>
<th>per oral</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Maximal volume (mL)</td>
<td>0.4</td>
<td>1</td>
<td>0.4</td>
<td>0.4</td>
<td>1</td>
</tr>
<tr>
<td>Mouse and guinea pig</td>
<td>Needle size</td>
<td>-</td>
<td>25G, 3/4”</td>
<td>25G, 1”</td>
<td>25G, 1”</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Maximal volume (mL)</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>0.4</td>
<td>-</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Needle size</td>
<td>25G, 1”</td>
<td>21G, 1”</td>
<td>25G, 1”</td>
<td>25G, 1”</td>
<td>catheter no.9</td>
</tr>
<tr>
<td></td>
<td>Maximal volume (mL)</td>
<td>10</td>
<td>5</td>
<td>2</td>
<td>2</td>
<td>5-10</td>
</tr>
</tbody>
</table>

G= Gauge

Drugs can be delivered to patients using various methods. Few drugs are only effective if given in a particular dosage form. Other drugs may be given in certain dosage forms so as it can increase or decrease as well as localize their effects.

1. **Oral Administration.** Many currently available drugs can be given orally. The drug can be administered orally in the form of tablets, capsules, powders, solutions, or suspensions. Drugs given via the oral route is usually used to obtain a systemic effect. These drugs must go through the digestive tract and usually undergo first pass metabolism.

2. **Parenteral Administration.** The term parenteral literally means to avoid intestinal (GI). Thus, administration of a drug means delivery directly into the body. In this route of administration, there is no absorption process in the gastrointestinal tract (DIT) before the drug effect takes place. Thus, this route has a faster onset of action than other routes of administration. Parenteral products should be sterile (free of microbial contamination). Parenteral route has its disadvantages: pain, discomfort, and the injected drugs cannot be withdrawn from the body.

   a. **Intravenous.**Injecting drugs directly into the patient's vein is the fastest route of administration. This type of administration route provides the most rapid onset of action.

   b. **Subcutaneous (Sub-Q / SC).** This route of administration involves injection of a drug under the skin into the fat layer, but not into the muscle. This drug is absorbed rapidly. Insulin is usually administered subcutaneously.
c. **Intraperitoneal (IP).** Although this method is rarely used clinically, this method is always used to deliver drugs in small animals. The muscle wall in the peritoneum (abdomen below) is very thin and has a lot of intestinal vascular blood vessels. This means that the injection at that area will cause a little pain, but the drug is easily absorbed into the circulatory system. Moreover drugs that are irritants and with large volume can be injected compared with other ways of administration.

**IV. SCREENING METHODS**

Dose is a certain amount of the drug that can be delivered to achieve a therapeutic effect. There are 5 types of doses, namely the minimum, common, maximum, toxic and lethal doses. To state the acute toxicity of the drug, commonly used size LD50 (lethal dose 50 medium) is a dose that kills 50% of a group of experimental animals. Likewise, as a measure of the effective dose (dose therapy), which is commonly used as a measure of ED 50 (median effective dose), i.e. the dose which gives a certain effect on 50% of a group of experimental animals. LD50 is determined by providing the drug at varied doses to a group of experimental animals. Each animal is given a single dose. After a certain period (e.g. 24 hours) most animal experiments will be dead. The doses provided to the animals (on the abscissa or x axis) is plotted against the percentage of animals that died (on the ordinate or y axis) on a graph paper. In pharmacodynamic studies, the therapeutic index of a drug is expressed in the following ratio:

\[
\text{Therapeutic index} = \frac{TD50}{ED50} \quad \text{or} \quad \frac{LD50}{ED50}
\]

LD50 is a result of the test (assay) and not a quantitative measurement. LD 50 is not an absolute value, and will vary from one laboratory to another, and different results could be obtained in the same laboratory each time the same experiment is executed (Ganiswara et al, 2007).

There are many commonly applied methods of calculating LD50, among other methods is Miller-Tainter, Reed-Muench method, and the Kärber method. In Miller-Tainter method, a logarithm-probit paper is used to calculate LD50. This paper has a logarithmic scale as abscissa and probit scale (this scale is not linear) as ordinate. In this graph paper, the mortality (in percent) at x axis is plotted against the logarithm of the dose (y axis). Reed-Muench method is based on the cumulative value of the number of animals alive and the number of dead animals. It is assumed that the dead animals with a certain dose will die in larger doses, and the alive animal will live with smaller doses. In Kärber method, its principle
is to use the average interval the number of deaths in each group of animals and dose
difference at the same intervals (Soemardji et al, 2009).

As previously mentioned, an ideal drug therapy should result in optimal therapeutic
effect to all patients without causing toxic effects even on a single patient (Ganiswara et al,
2007). The followings are procedures how to administer the drugs to the experimental
animals:

Subcutaneous
To inject mice subcutaneously put them on a table. Then place your left palm slowly
behind it and hold the skin at the nape of its neck with his thumb and forefinger. With the
right hand holding a syringe, stick a needle on the skin folds quickly. The tip of the needle
should move freely between the skin and muscle. If the length of the needle used was
appropriate, then the needle would not pierce too deep. Wiggle the needle with the index
finger and thumb to determine the position of the needle in the right place, then do the
injection. Pull the needle with your left hand, rub the injection site.

Oral
Solution of the drug can be administered orally using oral needle (catheter for rabbits).
For rats and mice, the animal is held perfectly and oral needle is inserted into the mouth
adjacent to the top of the roof of the mouth (palate). Push the needle slowly to esophagus and
do not force the entry. Upon entry into the mouth (approximately two inches down) the
animal will show the state of the choke. Oral needle can be adjusted by the amount of certain
animals.

Intraperitoneal
To inject the mice intraperitoneally, hold the neck skin of the animal with the index
finger and thumb. Perfect grip will stretch the skin in the abdomen. Inject at the lower
quadrant of the abdomen with a quick jab and do not hesitate. Push the needle into the part
where the needle does not penetrate the liver, kidney, spleen, or bladder, then press slowly.

Intravenous
Intravenous (IV) injection differs from one species to another. In mice, IV injection is
done at the tail vein. Therefore, blood vessels in mice are easy to locate, so an IV injection
can be done easily. The four tail veins located bilateral, ventral and dorsal and can be
developed (vasodilation) by touching a certain temperature at the tail portion (e.g. by putting mice into the warm water temperature 45-50°C), and the use of alcohol or by pressing the tip of its tail to facilitate injection. Animals are inserted in a mouse trap which resembles a tube with openings at both ends. Both ends are filled with cork which have a hole in its center. The tail end of the exit of the cork is held with the index finger and thumb of the left hand and injections are done with the right hand. It is better if we could shed light on the tail, it is intended to facilitate the sight of blood vessels clearly, also aims to heat the rats. When injected and no barriers are felt, at the injection site indicated that the needle has been entered correctly into a blood vessel and the plunger can be pressed easily. If the needle is not entered correctly on the blood vessels, the injecting area will give a pale region at the tip of the needle. It is better to use a piece of fine needle (Gauge 27.1 / 2 inches) and the injection could be started at the tail end so that several experiments can be done.

V. LUMINAL (PHENOBARBITAL)
Molecular pharmacology gamma-aminobutyric acid (GABA) chloride channel dependent receptor is one of the most reliable drug response machine in the body. Phenobarbital (5,5-phenyl-ethyl-barbituric acid) mimics GABA work. Phenobarbital is the first organic compound used as anticonvulsant. It acts by restricting the propagation of seizure activity and raise the threshold of stimulation. The main effects of barbiturates are CNS depression. All levels of depression can be achieved ranging from sedation, hypnosis, various levels of anesthesia, coma, and finally death. The hypnotic effect of phenobarbital can be reached within 20-60 minutes at a dose of hypnotics (Ganiswara et al, 2007).

VI. PRINCIPLE OF EXPERIMENT
Effect of varied doses, route of drug administration, and the individual animal variabilities can be observed following administration of phenobarbital in which level of hypnotic posed is reactive, slow motion, and sleep depends on the amount of the dose administered, route of administration, and physiologic variations of the experimental animals.

VII. EXPERIMENTAL METHOD
7.1 Tools
Electric scales, oral sonde mice, 1 ml syringes, stopwatch, 25 ml glass beaker, 10 ml Erlenmeyer

7.2 Materials
Distilled water, Luminal-Na concentration of 0.75%

7.3 Animal
The animals used are mice

7.4 Preparation of Na Phenobarbital Solution
Phenobarbital-Na with concentration of 0.75% is prepared by weighing 0.375 g of Na phenobarbital and dissolved in 50 ml of distilled water.

7.5 Experimental Procedure
1. The experimental animals are weighed and marked
2. The doses administered are calculated

Drug regimens and routes
- Mice 1: distilled water with dose of 1% BW administered orally.
- Mice 2: Luminal with dose of 80 mg / BW administered orally
- Mice 3: Luminal with dose of 80 mg / BW administered intraperitonially (i.p)
- Mice 4: Luminal with dose of 80 mg /BW s.c

Effect of Biological Variation
- Mice 1: weight 25 g Luminal with dose of 50 mg / kg orally.
- Mice 2: 35 g weight, Luminal with dose of 50 mg / kg orally.
- Mice 3: fasting, Luminal with dose of 50 mg / kg orally.
- Mice 4: without fasting, Luminal with dose of 50 mg / kg orally.
- Mice 5: males, Luminal with dose of 50 mg / kg orally.
- Mice 6: female, Luminal with dose of 50 mg / kg orally.

Dose, Response and Therapeutic Index
- Mice 1: control distilled water 1% BW orally.
- Mice 2: Luminal with dose of 50 mg / kg i.p
- Mice 3: Luminal with dose of 100 mg / kg i.p
- Mice 4: Luminal with dose of 200 mg / kg i.p
- Mice 5: 0.75% Luminal dose of 400 mg / kg i.p
The responses that occur within interval of 10 minutes for 90 minutes are observed and recorded and plot the response versus time on a graph paper.
REFERENCES


Report Experimental Data

The Title of experiment : 
Date of experiment : 
Group : 
Responser : 
Assistant : 

**Route of Drug Administration**

<table>
<thead>
<tr>
<th>No</th>
<th>TREATMENTS</th>
<th>TIME (min)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td></td>
<td>10 20 30 40 50 60 70 80 90</td>
</tr>
<tr>
<td>1</td>
<td>Control (distilled water) orally</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Luminal dose of 80 mg / kg orally</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Luminal dose of 80 mg / kg i.p</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Luminal dose of 80 mg / kg iv</td>
<td></td>
</tr>
</tbody>
</table>

**Dose, Response, and Therapeutic Index**

<table>
<thead>
<tr>
<th>No</th>
<th>TREATMENTS</th>
<th>TIME (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10 20 30 40 50 60 70 80 90</td>
</tr>
<tr>
<td>1</td>
<td>Control (distilled water) orally</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Luminal dose 50 mg/Kg BW i.p</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Luminal dose 100 mg/Kg BW i.p</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Luminal dose 200 mg/Kg BW i.p</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Luminal dose400 mg/Kg BW i.p</td>
<td></td>
</tr>
</tbody>
</table>
### Effect of Biological Variations on Drug Dose

<table>
<thead>
<tr>
<th>No</th>
<th>Treatments</th>
<th>RESPONSE</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>1</td>
<td>Mice 1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Mice 2</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Mice 3</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Mice 4</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Mice 5</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>Mice 6</td>
<td></td>
</tr>
</tbody>
</table>

Information:

1.1. Normal
1.2. Scratching (reactive)
1.3. Slow motion
1.4. Sleep
i.p = intra peritoneal
REFERENCES


SCORE :

Assistant, Medan, ________________

Student, (________________) (______________________)
GRAPHICS EXPERIMENT
ROUTE OF DRUG ADMINISTRATION, DOSAGE, RESPONSE AND INDEX THERAPY, BIOLOGICAL EFFECTS OF VARIATION ON DRUG DOSAGE
CHAPTER 2. ANALGESICS DRUGS ACTIVITY

I. SPECIFIC INSTRUCTIONAL OBJECTIVE

Upon completion of this experiment, students can evaluate the analgesic effects of drugs.

II. INTRODUCTION

Pain is an uncomfortable sensory and emotional feeling, related to (threatening) tissue damage. Pain in general is a symptom that serves as a hint of the danger of disruption in tissues such as inflammation, microorganism disease or muscle spasms. Pain caused by mechanical, chemical or physical stimulation (heat, electricity) can cause tissue damage where the stimulation causes the release of chemicals (eg: Bradykinin, Prostaglandins, ATP, protons) that stimulate receptors in patients.

Analgesics are substances that reduce or eliminate pain without losing consciousness. Based on its pharmacological work, analgesics are divided into 2 (two) major groups, namely peripheral analgesic (non narcotics) and narcotic analgetic. Peripheral analgesics (non-narcotics) consisting of drugs that are not narcotic and do not work centrally. While special narcotic analgesics are used to relieve severe pain, such as fractures and cancer.
Based on the process of occurrence, the pain can be overcome in several ways, namely:

a. Peripheral analgesics, which inhibit the formation of stimuli at peripheral receptors
b. Local analgesics, which inhibit the channeling of stimuli in the sensory nerves
c. Central analgesics (narcotics), which block the central nerve pain in the central nervous system (CNS) with general anesthesia
d. Tricyclic antidepressives, which are used in cancer and nerve pain
e. Antiepileptic, which increases the number of neurotransmitters in the synapse chamber in pain

Pain perception is a difficult situation to define or measure. It is a subjective phenomenon, so it can not be known how the image of experimental animals is experiencing pain. Most techniques involve the use of nociceptive tests in which painful stimuli, mechanically or electrically are used to produce pain.

The usual method is the Janssen and Jageneu hot plate method (1975). In this method the animal is placed slowly onto a hot plate with a fixed temperature of 550°C. Response time (usually 4-10 seconds for normal state is calculated as the initial distance of time the animal puts its feet on the plate and time is recorded when the animal starts licking feet or jumps to escape from the heat). Animals that did not show a response within 30 seconds were not used in the experiment.

Another method is to use a chemical compound such as 3% acetic acid. This acetic acid as a stimulus for the pain caused. The pain of giving acetic acid can be seen from the existing stretching from observation to mice (animals). This stretch is calculated starting if the mice stretch her legs back and pressing her stomach down. This stretch is calculated 1, and so on. So that the end of the specified time will get the total number of stretch animals in a particular time.

In the experiment, three methods were used to describe the perception of pain, the acetic acid method as a peripheral pain stimulus, the hot plate method, and the heat method using infra-red (IR) as the central pain stimulus.

### III. METHOD

#### 3.1 Tools

a. Electric Scale
b. 1 ml Syringe
c. Stopwatch
d. 25 ml Beaker glass
3.2 Materials

- a. Mice
- b. Aquadest
- c. Acetid Acid 3%
- d. Antalgin 2%
- e. Morphine SO\textsubscript{4} 0.1%

IV. MAKING OF DRUG SOLUTION

a. Morphine SO\textsubscript{4} 0.1 %
Morphine 0.025 g is weighed, then diluted with aquadest in a 25 ml flask up to the mark line.

b. Antalgin (Methampiron HCl) 2%
Antalgin 1 g is weighed, diluted with Aquadest in a 50 ml flask up to the mark line.

c. 3% acetic acid
The concentration of 3% is made by dissolving 10 ml of acetic acid in 15% aquadest in 50 ml flask.

V. PROCEDURE

Acetic Acid Method
1. Animals are weighed and marked
2. Dose administration are calculated:
   - Mice 1: Control aquadest at the dose of 1% of body weight (i.p)
   - Mice 2: Morphine SO\textsubscript{4} [ ] 0.1%, 10 mg/kg BW (i.p)
   - Mice 3: Morphine SO\textsubscript{4} [ ] 0.1%, 15 mg/kg BW (i.p)
   - Mice 4: antalgin [ ] 2%, 300 mg/kg BW (i.p)
   - Mice 5: antalgin [ ] 2%, 400 mg/kg BW (i.p)
3. After 30 minutes, 3% acetic acid at a dose of 1% body weight is injected (i.p).
4. The amount of stretching interval from 10 minutes to 90 minutes is observed and calculated
5. Plot a Graph based on the amount of stretching vs time
6. Data is then analyzed statistically.

Hot Plate Method
1. Animals are weighed and marked
2. Dose administration are calculated:
   - Mice 1: Control aquadest at the dose of 1% of body weight (i.p)
   - Mice 2: Morphine SO4 [ ] 0.1%, 10 mg/kg BW (i.p)
   - Mice 3: Morphine SO4 [ ] 0.1%, 15 mg/kg BW (i.p)
   - Mice 4: antalgin [ ] 2%, 300 mg/kg BW (i.p)
   - Mice 5: antalgin [ ] 2%, 400 mg/kg BW (i.p)

3. The animals are placed onto a hot plate at 55°C.

4. The time when the animals start to lick its feet from the 10 minute to 90 minute is observed and calculated.

5. Then plot a graph based on period of response vs time

6. Data is analyzed statistically.

**Infrared Heat plate method (IR)**

1. Animals were weighed and marked

Dose administration are calculated:
   - Mice 1: Control aquadest at the dose of 1% of body weight (i.p)
   - Mice 2: Morphine SO4 [ ] 0.1%, 10 mg/kg BW (i.p)
   - Mice 3: Morphine SO4 [ ] 0.1%, 15 mg/kg BW (i.p)
   - Mice 4: antalgin [ ] 2%, 300 mg/kg BW (i.p)
   - Mice 5: antalgin [ ] 2%, 400 mg/kg BW (i.p)

3. The animals are put into the box, then direct the IR heat right into the animal's foot

4. The time interval from the 10 minute to 90 minute is observed and recorded

5. Plot the graph of response period vs time graph

6. Data is then analyzed statistically.

**VI. Graph**

Graph Total Stretching vs Time

Total Stretching

![Graph Total Stretching vs Time](image-url)
### VII. Report Experimental Data

**Title**: 

**Date**: 

**Group**: 

**Responder**: 

**Assistant Supervisor**: 

<table>
<thead>
<tr>
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<td>After 30 minutes, each mouse is injected at a dose of 3% acetic acid 1% BW (i.p)</td>
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<td>Antalgin 250 mg/kg BW</td>
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<tr>
<td></td>
<td>Mice 1</td>
<td>Put in the box, Then direct the IR heat right on the feet of the mice</td>
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<td>Control (NaCl [0.9%] 1% BW)</td>
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<td>Mice 2</td>
<td>Mice placed on hot plate 55 °C</td>
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<td>(Morphine 15 mg / kg BW)</td>
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<td>Mice 3</td>
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<td>(Antalgin 400 mg/kg BW)</td>
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<td>(Antalgin 400 mg/kg BW)</td>
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VIII. DISCUSSION
REFERENCES


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ANALGESIC EXPERIMENT GRAPHIC
CHAPTER 3. ANTIPYRETIC ACTIVITY OF THE DRUG / TEST PREPARATION

I. Specific Instructional Objective

After completing this experiment, students can evaluate the Activity of Antipyretic of the Drug.

II. Introduction

Fever or the body temperature increase generally occurs due to infection. Toxins produced by microorganisms will disrupt the body heat regulation system in the hypothalamus. In addition, can be affected by toxins from microorganisms. The body heat regulation system can also be affected by other substances that are toxic that enters the body. At temperature above 37°C lymphocytes and macrophages become more active, and if the temperature exceeds 40-41°C could occur in critical situations become fatal because can not be controlled anymore by the body.

Based on the concepts above, developed ways to conduct experiments testing the effectiveness of a drug antipyretic. Dinitrophenol originally used as a hot-forming compounds and drugs for weight loss. Dinitrophenol known to be extremely toxic and can cause cataracts.

Antipyretic is compounds that can decrease the body temperature in a state of fever, one of the example is paracetamol. Antipyretic used extensively in control pyrexia caused by some viral diseases, malaria, malignancy, tissue damage, inflammatory and the level of other diseases. To evaluate the antipyretic in overcoming the fever then conducted animal experiments using fungal injection Brewer or Lipopolysaccharide-lipopolysaccharide.

Antipyretic Test of Rat:

In rats given injections subcutaneously suspension Brewer fungus produces significant pyrexia which can be overcome by the drugs were clinically effective antipyretic.

Antipyretic Test of Rabbit:

Rabbits are very sensitive to the effects of lipopolysaccharide-lipopolysaccharide pyrexigenik who was conceived by gram-negative bacteria- E.coli is administered intravenously. Lipopolysaccharide fraction which cause an increase in body temperature of 1°C or more at a dose 0,1-0,2 µg/kg be used for further research.
III. Experimental Method

3.1 Tools
   a. Rectal Thermometer
   b. Animal Electric Scale
   c. Stopwatch
   d. Syringe (1 ml, 2 ml dan 5 ml)
   e. Oral Sonde

3.2 Materials
   a. 0.1 N NaOH Solution
   b. CMC
   c. Paracetamol
   d. Alcohol 70%
   e. Vaseline
   f. 2,4 Dinitrophenol (DNF)

IV. Making Of Drug Solution

a. Injection 2,4-dinitrofenol 0.5%
   Procedure:
   A total of 500 mg of 2,4-dinitrophenol is weighed, and then put in a 100 ml flask, then added 0.1 N NaOH solution little by little until late. Aquadest was added to the line mark, pH is set at 6, the solution is filtered, and the first 5 drops is removed and droplets subsequently accommodated.

b. Paracetamol suspension of 10%
   Procedure:
   CMC as much as 0.125 g sown into the porcelain dish containing hot aquadest as much as 1/3 of water available. After 30 minutes, the mixture is stirred until a homogeneous mass is obtained. Paracetamol 2.5g is crushed in a mortar until smooth, mucilago CMC added little by little while crushed until homogeneous. The remainder is added to 25 ml of aquadest, crushed back.
V. Procedure

1. Animals are weighed and marked.
2. The temperature of 3 rats are measured with a rectal thermometer with an interval of 5 minutes for 3 times.
3. 2, 4 dinitrophenol 0.5% at the dose of 5 mg/KgBW is intramuscularly administered.
4. The elevation of body temperature of mice is measured in interval of 5 to 20 minutes.
5. Doses are calculated and administered:
   a. Rat 1: CMC Na suspension 0.5% orally.
   b. Rat 2: paracetamol suspension of 10% at the dose of 400 mg/KgBW orally.
   c. Rat 3: X drug at the dose of 400 mg/Kg BW orally.
6. Temperature change that occur with an interval of 5 minutes to 50 minutes is measured.
7. Temperature vs. time graph is made.

VI. Graph

*Temperature vs. Time Graph*

*Temperature* \(^\circ\)C

![Graph Image]

Information:

a. Each line of the graph is made in various colors (for each animal)
b. The scale of the graph is adjusted
VII. Report Experimental Data

Title : 
Date : 
Group : 
Responder : 
Assistant Supervisor : 

A. The Average Temperature of Rats

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<td>2.</td>
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<td>Mean</td>
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<td>3.</td>
<td>Rat 3 (X Drug)</td>
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### B. Temperature After DNF Administration

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<td>2.</td>
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<td>Rat 3 (X Drug)</td>
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C. Temperature After Drug Administration

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<td>2.</td>
<td>Rat 2 (Paracetamol oral suspension)</td>
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<td></td>
<td>Rat 3 (X Drug)</td>
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VII. Discussion
REFERENCES

SCORE:

Assistant

Student

Medan, ____________
CHARTS TRIAL ANTIPYRETIC
CHAPTER 4. ANTI-INFLAMMATORY ACTIVITY OF DRUG

I. Specific Instructional Review
After completing this experiment, students will be able to evaluate the activity of anti-inflammatory drugs.

II. Introduction
Inflammation is a normal protective response to injured tissue caused by physical trauma, damaging chemicals, and microbiological substances. Inflammation is an attempt to inactivate or destroy microorganisms that attack, removing irritants, and adjusting the degree of tissue repair. If healing is completed, the inflammation process usually subsides. Inflammation is triggered by the release of chemical mediators, (such as prostaglandins, histamine and leukotrienes) and cell migration (which is triggered by pro-inflammatory cytokines) (Myczek et al. 1997). Inflammatory process is known by the five major signs: heat (color), redness (rubor), pain (dolor), swelling (tumor), and loss of function (Eales 2003).

Based the long of happening, inflammation can be divided into two types, namely: acute inflammation and chronic inflammation. Acute inflammation is the earliest defense reaction of the body tissues to the agent destroyer, and end after a few hours or days. The cause of acute inflammation is such as microbes, hypersensitivity reactions, chemical substances, physical trauma and tissue damage. The immune cells which have a role in this reaction are neutrophils, eosinophils and mast cells (Shell 1987). While chronic inflammation is the body inflammatory reaction that occurs in a longer period of time. Chronic inflammation involves many types of immune cells, such as mononuclear phagocytic cell and T cell lymphocytes (Stephenson 2004).

Prostaglandin is a major chemical mediator involved in the inflammatory process, in the other of chemical mediators, and becomes the target of anti-inflammatory drugs. Arachidonic acid is the main precursor prostaglandin. Arachidonic acid is released from phospholipids by the action of phospholipase A2 and other acyl hydrolases. Furthermore, biosynthesised again with the help of cyclooxygenase (COX) into eicosanoids. There are two major isomers of COX which act in the biosynthesis of prostaglandins, COX1 and COX2. COX1 is found everywhere, while the second is induced in response to inflammatory stimuli. Prostaglandins
and its metabolites which are produced endogenously within the tissue to work as a sign of locally for adjusting responses specific cell type (Mycek et al. 1997).

III. Screening Method

_In vivo_ method, the animal model of inflammatory is used in determining the activity of compound or drugs ingredients as anti-inflammation. Animal model of inflammation can be obtained by injecting it in intraplantar test animal (rat or mice) with inducer such as carrageenan, a foreign antigen and arachidonic acid, which can trigger the inflammatory process (characterized by swelling in the feet of animals inflammatory) (Blank et al. 2004). Materials inducer of inflammatory trigger the complex inflammatory mechanism, involve many mechanisms, including the release of mediators biochemistry, such as prostaglandins, histamine, bradykinin, pro-inflammatory cytokines, as well as increased migration of cells of leukocytes into the inflammation site (Chiang et al., 2005). Furthermore, animal model of inflammation is treated by the test preparation or drug compound whose the dose that has been prescribed. Anti-inflammatory activity can be determined by measuring the swelling in the feet the animal model within a certain time interval, using a tool pletismometer. Reduced swelling on the sole test animal indicates anti-inflammatory activity.

IV. Antiinflammatory Drugs

Based on its mechanism of action, in general anti-inflammatory can be divided into two classes of drugs, which is a non-steroidal anti-inflammatory (NSAID) and anti-inflammatory steroid.

1. Non-steroidal Anti-inflammatory (NSAID) drug group works by inhibiting (inhibition) cyclooxygenase enzyme responsible for prostaglandin biosynthesis, but does not work on the inhibition of lipoxygenase enzyme. Cyclooxygenase enzyme has several isomer, such as COX1, COX2 and COX3, based on this same class of non-steroidal anti-inflammatory drug can be divided into selective NSAID and non-selective NSAID. Selective NSAID work by inhibiting the COX isomer, such as COX2, examples of these drugs are celecoxib. While the non-selective NSAID work by inhibiting COX all isomers, examples of this class of drugs are aspirin (drug prototype), indomethacin, diclofenac (Katzung, 1992).

2. Anti-inflammatory steroid

This class of drug works by inhibiting the enzyme phospholipase A2, which is responsible for the release of arachidonic acid (a precursor of prostaglandin) on the cell membrane. Example of this class of drug is Prednisone (Mycek et al. 1997).
V. Tools And Materials

5.1 Tools
Syringe, oral sonde, manual or digital Pletismometer.

5.2 Materials
1% Carrageenan solution in aquadest (made the day before the experiment), Suspension of CMC Na as a control, Dexamethasone drug suspension 0.0015 % with dose 0.045 mg/kg BW

5.3 Animal Model
Rats.

5.4 Procedure
1. Rats were fasted (fixed by drinking water) since ± 18 hours before the experiment
2. Rats were weighed and then given a mark on the left rear leg joints for each rats.
3. The volume of foot were measured by dipping a toe that has been marked to the extent that the mark has been supplied to the pletismometer, and viewed the height of liquid on the appliance (if using pletismometer manual) or the value which shown on the screen (if using digital pletismometer). This value is expressed as the initial volume (V0).
4. Rats were given the drug suspension Dexamethasone 0.0015% with dose 0.045 mg/kg BW, suspension of X drug 360 mg/kg BW, suspension of CMC Na for the control rats orally.
5. 30 minutes after drug administration, 1% carrageenan solution is injected with 0.05 ml volume to the left rear foot of each rat.
6. 30 minutes later, the volume of which has been injected by carrageenan is measured and recorded. The measurement carried out for 3 hours with intervals of 30 minutes.
7. Record the observations in the table, and for each rats, calculate the percentage of inhibition of inflammation and inflammation that occurs for each time point (30 minutes, 60 minutes, 90 minutes and so on) using the formula:

For Inflammation Percentage (% R) :

\[
\% R = \left( \frac{V_t - V_0}{V_0} \right) \times 100 \%
\]

Information :
Vt = Volume of foot at time
V0 = Volume of foot early
For Inhibition of Inflammation Percentage (% IR) :

\[
\%IR = \frac{\left(\% R\ Control - \% R\ Drug\right)}{\% R\ Control} \times 100 \%
\]

Information

% Control = Percentage of inflammation control group
% Drug = Percentage of inflammatory group of drugs

8. Based on the data obtained, draw graphs the percentage of inflammation and the percentage of inflammatory inhibition in time-dependently
VI. Report Experimental Data

Title of Experiment : 
Date of Experiment : 
Group : 
Responder : 
Supervisor Assistant : 

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<td></td>
<td>Vt</td>
<td>% R</td>
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<td>A Drug Rat</td>
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<td>3</td>
<td>B Drug Rat</td>
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GRAPHS OF ANTIINFLAMMATORY EXPERIMENT
CHAPTER 5. ACTIVITIES OF ANTIDIABETIC DRUGS

I. Specific Instructional Objectives
After finishing this experiment, the students can evaluate the activity of antidiabetic drugs.

II. Introduction
Diabetes Mellitus is a group of metabolic disorders of fat, carbohydrate, and protein metabolism that results from defects in insulin secretion, insulin action (sensitivity), or both.

Secretion of insulin is stimulated by a large number of endogenous and exogenous substances. Glucose is one exogenous substances that become the main determinant of β-cell function in synthesis and release insulin. Glucose in the blood will enter the β-cells mediated by glut-2. Furthermore, glucose undergo metabolic processes, ultimately leading to the opening of voltage-gated calcium channels. Increasing intracellular calcium, stimulates exocytosis followed the release of insulin and other components into circulation (Lawrence, 2005).

Then insulin binds to the receptor on the surface of cell in the target tissue. The target tissue is important for the regulation of glucose homeostasis are liver, muscle and fat. In addition to that, insulin also works on blood cells, brain cells and gonadal cells. The interaction between insulin and the receptor generates a signal that is transmitted into the cell to activate various anabolic pathways and inhibit catabolic processes. Glucose transport into skeletal muscle and adipose cells is mediated by glut-4. Insulin also increases glucose entry into heart cells. Glucose in the cells can then be metabolized in different ways. In skeletal muscle and liver, glucose is stored as glycogen (glycogenesis) for reusable (glycogenolysis). In the fat cells, glucose is metabolized into Acetyl-CoA which is then used to synthesize fatty acids. Esterification of fatty acids with glycerol to produce triglycerides which are a form of energy storage.

III. Screening Method
The animal of Diabetes Mellitus is used to validate a variety of herbs that is thought to have potential as an antidiabetic. In in vivo method, animal models of Diabetes Mellitus can be obtained by inducing animal pharmacologically, surgery or genetic engineering. Some animals can be used in experiment, namely rodents and non-rodents. But largely the studies is conducted in rodents such as rats and mice. Non-rodents animals which is better to use is rabbit, and it is claimed to be a better animal model (Frode and Medeiros, 2008, the Scientific Working Group Phyto Medica, 1993; Rees and Alcolado, 2005)
Some method can be applied to create diabetes to the animal test, for example using glucose in glucose tolerance test, Streptozotosin as well as Alloxan Monohydrate. Streptozotosin can create more stable and permanent diabetes in animal model (Frode and Medeiros, 2008). Alloxan is a glucose analog that is toxic. When it is administered to the animals test it will accumulate selectively in pancreatic $\beta$ cells and produce free radicals. The formation of free radicals can cause damaging on $\beta$-cell and causing disruption of insulin production (Lenzen, 2008).

Induction diabetogenic compound in animal experiments can be carried out parenterally such as intravenous, intraperitoneal, and subcutaneous. The dose required to induce diabetes mellitus depend on the route of administration, the type of animal and nutritional status. Most commonly dose of Alloxan which is used on mice is 65 mg / kg of body weight by intravenous. For intraperitoneal administration, it should be higher, to make it more effective. Some researchers use dose of 120 mg / kg body weight to 160 mg / kg body weight (Lenzen et al., 1996; Federiuk, et al., 2004).

The time required from start of induction to the occurrence of diabetes is approximately 3 to 5 days, depending on the dose of alloxan and endurance of test animals (Frode and Medeiros, 2008). Before induction, animals were fasted for 8-12 hours, or even up to 16 hours (Katsumata et al., 1992; Federiuk, et al., 2004; Rees and Alcolado, 2005).

**IV. Antidiabetic Drugs**

Treatment of diabetes mellitus can be done non-pharmacological, pharmacological or a combination of both. In non-pharmacological is the low-carbohydrate diet and exercise. Pharmacologically is the provision of drugs both insulin and non-insulin.

The following medications are included in non-insulin drugs that are often used by people with Diabetes Mellitus.

A. Oral Hypoglycemic Group

Oral hypoglycemic drugs are drugs that is able to lower blood glucose level by stimulating insulin release from $\beta$-cells of the pancreas. These drugs has the potential activity to lower blood glucose levels to below normal levels. Groups which are included in a class of oral hypoglycemic are sulfonylureas and meglitinid (Lawrence, 2005).
B. Anti Hyperglycemia Group
Anti hyperglycemia is a drug that lowers blood glucose levels and less likely to cause hypoglycemia. This group of drugs with different mechanisms of action of oral hypoglycemic group. There are 3 groups belonging to this group, they are Biguanide, Thiazolidinediones and α-glucosidase inhibitors (Lawrence, 2005).

C. The New Antidiabetic Group
The new antidiabetic groups is an incretin mimetics and DPP IV inhibitor. Incretin mimetic is a new antidiabetic group which can mimic the effects of endogenous incretin hormone that indicates multiple glucoregulator activity. In the end, these drugs can stimulate insulin secretion while inhibiting the release of glucagon, resulting in a decrease in blood glucose levels. Two classes of drugs that qualify as incretin is a glucagon-like peptide-1 (GLP-1) and gastric inhibitory peptide (GIP). Both GLP-1 and GIP are rapidly inactivated by the enzyme dipeptidyl peptidase-4 (DPP-4). DPP-4 inhibitors enhance the incretin GLP-1 concentrations in the blood by inhibiting its degradation by DPP-4.

V. Method
5.1 Tools
Syringe with oral sonde, glucotest, mice restrainer.

5.2 Materials
Glucose monohydrate, CMC-Na, Glibenclamide, strip test, plant extracts.

5.3 Animals
Mice age 2-3 months.

5.4 Procedure
- Mice were fasted (not eaten but still drinking) for 1 day, weighed and blood glucose levels were measured
- Furthermore, mice were divided into three groups, namely:
  a. The negative control group, which is given CMC 0.5% as much as 1% body weight
  b. The test group, which is given the plant extract.
  c. The positive control group, which is given Glibenclamide 0.01% 0.45 mg / kg
- After 30 minutes, they are given a solution of glucose 3 g / kg body weight per oral
- Withdrawn blood from the tail and measured blood sugar levels at minute 30, 60, 90 and 120 after loading glucose.
- Analysis of blood glucose levels between the test group, negative control group as well as positive control group.

**VI. Report Experimental Data**

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VII. Discussion
REFERENCES

Federiuk, I.F., Casey, H.M., Quinn, M.J., Wood, M.D. dan Ward, WK. (2004) : Induction of Type-1 Diabetes Mellitus in Laboratory Rats by Use of Alloxan: Route of Administration, Pitfalls, and Insulin Treatment, Comparative Medicine, (54), 252-257.


Score : 

Assistant, 

Medan, ____________

Student,

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GRAPHICS EXPERIMENT
ACTIVITIES OF ANTIDIABETIC DRUG
I. SPECIFIC PURPOSE INSTRUCTIONAL
After finishing this experiment, students can evaluate the activity of diuretic drugs or pharmaceutical test.

II. INTRODUCTION
Diuretic is a drug which act on the kidney to increase the secretion of water and salt. The main function of diuretic is to overcome oedema, which can mobilize body fluid means change the balance of the fluid returned to normally. Besides to overcome oedema, diuretic also effective to the people who has hypertension, diabetes insipidus, hyponatremia, nephrolithiasis, hypercalcemia, and glaucoma. Although all diuretics generally can increase excretion of water and salt to decrease the extracellular fluid, but its mechanism is different.

III. DIURETIC DRUGS
- **Organic mercury**: Clormerodrin, Meralurid, Mercaptomerin
- **Derivatives of xanthine**: Caffeine, Theophylline, Theobromine
  
  **Mechanism of action:**
  Xanthine derivative is a mild to moderate diuretic. These compounds work by increasing the supply of blood to nephron notably in the area of renal medulla. At the same time vasa afferen resistance will be reduced much more than vasa efferen. So, glomerulus filtration rate will be greater. Xanthine derivative, may be the only one diuretic that can increase glomerulus filtration rate and works at least due to by increasing formation of primary urine. Greater blood supply on renal medulla will causes more diuresis. Longer application will decrease its efficacy, so the xanthine derivatives no longer use as diuretic.

- **Osmotic diuretics**: Mannitol, Sorbitol, Glyserin, Urea, Isosorbide
  
  **Mechanism of action:**
  This compound is inert, after filtration in glomerulus, it will not reabsorbed by tubule. Based on osmotic pressure, this compound will inhibit reabsorption of water in the lumen tubule, while sodium will reabsorbed. The reabsorbsion of sodium is less because the different concentration of sodium happened so fast when the sodium in the lumen less than on cell, so there are will be more sodium inhibit. Thus, diuresis will be increase. The excretion of electrolyte increase slightly by these compounds.
- **Inhibitors of carbonic anhydrase**: Acetazolamide, Diclorfenamide, Metazolamide
  
  **Mechanism of action:**
  These drugs primarily work in the proximal tubule and the other site in the collecting duct by inhibit the enzyme carbonic anhydrase, thus decrease tubule reabsorption from sodium ion, cause the amount of ion $\text{H}^+$ that entered to the lumen is less. The consequence is happened to increased sodium ion, potassium and hydrogen carbonate through kidney and also water excretion. Losing base will causes acidosis in the blood. By this, works of inhibitors carbonic anhydrase decrease fastly.

- **Thiazide Diuretics** *(Inhibitor Na$^+$ dan Cl$^-$ Symport)*
  Derivatives of dihydrobenzothiazide: Hydrochlorothiazide, Trichlormethiazide, Buthiazide, Polythiazide, Bendroflumethiazide

- **Sulfonamide diuretics analogy of thiazides**: Mefrusida, Chlopamide, Chlortalidon, Xipamide
  
  **Mechanism of action:**
  These drugs inhibit symport Na$^+$ - Cl$^-$ may be, inhibited the reabsorption of sodium and chloride in distal tubule (primary site of action) and proxima tubule (weak action in inhibitors carbonic anhydrase). This symport was regulated by aldosteron.

- **The Diuretic Loop of Henle** *(Inhibitors Of Na$^+$–K$^+$–2Cl$^-$ Symport)*
  The Diuretic Loop of Henle the type of furosemide: Furosemide, Bumetanide, Piretanide
  
  Another group diuretic from Loop of Henle: Ethacrynic acid, Etozoline, Muzolimine
  
  **Mechanism of action:**
  At the boundary between the inner and outer stripes of the outer medulla, the thin limb of Henle’s loop begins. The NaCl transport system in the luminal membrane of the thick ascending limb is a Na$^+$/K$^+$/2Cl$^-$ cotransporter. This transporter is selectively blocked by diuretic agents known as “loop” diuretics (these drugs are the most efficacious diuretic agents available). These drugs entered to the tubule liquid to blocked the transporter of NaCl transport happened by active secretion of proximal tubule. This tells us, for patients with renal insufficiency that influence the secretion need more doses and the onset of action of the drugs took more duration.

- **Potassium-Sparing Diuretic**
  Antagonize of aldosteron: Spironolactone, Kanrenone (active metabolite), Kanrenoat potassium, Eplerenone
Mechanism of action:
Spironolactone (or Kanrenone) acts as a competitive antagonist to aldosterone to the cytoplasm receptor in the last-distal tubule and also in collecting tubule. Thus, aldosterone couldn’t entered the nucleus to band with the receptors and couldn’t production protein that can open sodium’s channel in the luminal. Finally, absorbed decrease and the excretion of potassium also decreased.

- **Derivatives of Cycloamidin**: Triamterene, Amiloride
  Mechanism of action:
  These drugs blocked sodium’s channel in the last-distal tubule and also in collecting tubule. Besides, another assumption that these drugs also act in potassium’s channel (cause secretion of K⁺ related to entered Na⁺) or in transporter to exchange the proton-sodium.

**IV. EXPERIMENT METHODS**

4.1 **Tools**
Metabolism cage, syringe, oral tube, measure-glass, vial

4.2 **Materials**
Furosemide, Extract, CMC, aquadestilation

4.3 **Animal Sample**
Mice (wistar rats) age 4 months

4.4 **Procedure**
1. Mice were fasted for 1 day
2. Measure their weight and and then divide it into three groups, namely:
   - I: Control group, is given CMC Na 0.5%
   - II: Sample group, is given extract
   - III: Comparison group, is given Furosemide 3.6 mg/kg body weight
3. Give *loading* NaCl 3 mL/kg body weight
4. Wait until 2 hours, and then measure volume of the urine
5. Analyze data based on statistic.
## DATA EXPERIMENT

**Title**:  
**Date**:  
**Group**:  
**Responser**:  
**Assistant supervisor**:  

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V. DISCUSSION
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Assistant,

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CHAPTER 7. THE ACTIVITY OF THE CENTRAL NERVOUS SYSTEM

DRUGS

I. SPECIFIC INSTRUCTIONAL OBJECTIVE

After completing this experiment students can evaluate the activity of drugs on the central nervous system.

II. INTRODUCTION

Drugs that work on the central nervous system (CNS) is one of the first drugs discovered by primitive people and still widely used as pharmacological substances until now. Some of these drugs are addictive and have many disadvantages for personal, social or economic aspects, thus it is necessary to regulate the use of these drugs. The mechanism of action of various drugs on the CNS cannot always be explained because the causes of the curable diseases (such as schizophrenia, anxiety) are not yet entirely known. CNS drugs work on specific receptors that regulate the transmission of synapses. Some medications such as general anaesthetics and alcohol may work in non-specific membrane (although this exception is not fully accepted) but working without going through these receptors lead to the changes in the transmission of the synapses. CNS drugs are the most important tool for studying the physiological aspects of CNS such as the generation of stimulation and long term memory storage. CNS drugs that have clinical benefits have brought to hypothesis concerning the mechanism of the disease. For example, information about the mechanism of antipsychotic drugs to their receptors provide the basic hypothesis about the pathology of schizophrenia. The study of some effects of agonist and antagonists gamma amino butyric acid (GABA) receptor provides new concept of diseases including anxiety and epilepsy.

III. SCREENING METHOD

Isoniazid may cause convulsion by inhibiting the synthesis of GABA (gamma amino butyric acid). GABA is a inhibitory neurotransmitter which can cause hyperpolarization of the central nervous system (Harahap and Hadisahputra, 1999), so that when the amount of GABA decreases, convulsion effects will occur. In more details, the enzyme is inhibited by glutamate decarboksilase pyridoxal 5 phosphate which is a co-factors for the enzyme, resulting in a decrease in the amount of GABA (Vasu and Saluja, 2005). Diazepam is a muscle relaxant that works in the CNS and can be used to overcome spasm due to strychnine.
IV. Method

4.1 Tools
Syringe with oral sonde, mice restrainer, stopwatch.

4.2 Materials
Isoniazide, Diazepam, NaCl 0.9%, distilled water, and CMC Na 0.5%.

4.3 Animal
Mice at 2-3 months of age are used in this experiment.

4.4 Procedure
a. The mice is weighed, recorded and marked on its tail.
b. The doses is then calculated as the doses below:
   - Mouse 1 : Control NaCl 0.9% at the -dose of 1%/bb (i. p)
   - Mouse 2 : Diazepam 0.5% at the dose of 20 mg/kg BW (i.p)
   - Mouse 3 : Diazepam 0.5% at the dose of 25 mg/kg BW (i.p)
c. Symptoms arised in the mice are observed
d. After 1 hour, each mouse is injected with Isoniazide 2% at the dose of 400 mg/kg BW
   intraperitoneally, then the onset of convulsion (beginning of the seizure), the duration
   of protection for 2 hours and the number of deaths during the 2 hours are observed.
e. Graph response vs time is made.

V. Graphs
Notes:
   i. Every line in the graph is drawn indifferent colors (for each animal)
   ii. The scale of the graphs should be adjusted
VI. Experimental Data Report

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Date of The Experiment : 
Group : 
Responder : 
Supervisor Assistant :

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VII. Discussion
REFERENCES

Score: ___________________

Assistant, Medan, ________________

Student, ________________________
CHAPTER 8. THE ACTIVITY OF THE DRUG AGAINST THE PERIPHERAL NERVOUS SYSTEM

Drug Effects On Parasympathetic And Sympathetic Nervous System

I. Specific Instructional Objective
After completing this experiment, students can evaluate the activity of drugs that affect parasympathetic and sympathetic nervous system.

II. Introduction
Cholinergic drugs, also known as parasympathomimetic, any various drugs that resemble the parasympathetic nerve stimulation. However, some nerves that are anatomically belong to sympathetic nervous system are having acetylcholine as their neurotransmitter, that is why cholinergic is more appropriate than the term of parasympathomimetic.

Cholinergic drugs are divided into three groups:
1. Cetylcholine: including Acetylcholine, Metakolin, Karbakol, Betanekol
2. Anticholinesterase: including Eserin (fisostigmin), Prostigmin (neostigmin), Diisopropyl-flurofosfat (DFP), and insecticide (the organophosphate).
3. Alkaloids, such as Muscarine, Pilocarpine, and Acetylcholine.

Adrenergic drugs is also called sympatomimetic, which resemble the effect caused by sympathetic nerves (Gan, 2007).

An autonomous organ response against adrenergic excitation depend on the type of adrenergic receptor owned by the organ. For example, the radial muscle of the eye’s iris has α1 reseptor, it indicate that the stimulation of adrenergic nerve will cause a contraction (midriasis). The eye’s siliaris muscle has β2 receptor, then the adrenergic nerve stimulation cause relaxation (weak) (Gan, 2007). The eye is an example of an organ with many functions of the autonomic nervous system, which controlled various autonomic receptors. Muscarinic cholinomimetic lead to pupil circular constrictor muscle and siliaris muscle contractions.

III. Method
3.1 Tools
Animal Scales (rabbit), dropper bottle, stopwatch, flashlight (light), vernier caliper, LUV (magnifying glass)
3.2 Materials
Pilocarpine 1%, Atropine 1%

3.3 Animal
Rabbit, 1.5-2 kg body of weight

3.4 Procedure
a. Measure the diameter of the normal right eye and left eye of rabbits and the reflex against the light 3 times with an interval of 5 minutes.
b. Give 2 drops of Pilocarpine on the right and left eyes.
c. Observed the pupil’s diameter (both right and left eye) of the rabbit as well as reflex to the light in 30-minute with an interval of 5 minutes.
d. After 30 minutes, give the each eye 2 drops of Atropine
e. Observed the pupil’s diameter (both right and left eye) of the rabbit as well as reflex to the light in 30-minute with an interval of 5 minutes.
f. Made a graph of pupil diameter vs. time

IV. GRAPH

Right eye Diameter chart vs. Time

Diameter (mm)

Time (minute)
V. Report Experimental Data

Experiment title : 
Date of experiment : 
Group : 
Responser : 
Assistant supervisor : 

A. Control rabbit

<table>
<thead>
<tr>
<th>No</th>
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<th>Time (minutes)</th>
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<th>Right Eye</th>
</tr>
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<tr>
<td></td>
<td></td>
<td></td>
<td>D (mm)</td>
<td>Refleks</td>
</tr>
<tr>
<td>1</td>
<td>Normal</td>
<td>5</td>
<td></td>
<td></td>
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<td></td>
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<td>15</td>
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<td></td>
<td></td>
<td>Average</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Pilocarpin</td>
<td>5</td>
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B. Rabbit after treatment

<table>
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<tr>
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<td>Time (Minute)</td>
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<td>Right Eye</td>
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<td>30</td>
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<tr>
<td>3</td>
<td>Atropin Sulfat</td>
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</table>

**Information:**
+ = Slow
++ = Fast
- = No Reaction
VI. Discussions
REFERENCES

EXPERIMENT GRAPH
THE ACTIVITY OF THE DRUG AGAINST THE PERIPHERAL NERVOUS SYSTEM
CHAPTER 9. THE ACTIVITY OF DRUG/PHARMACEUTICAL TEST TO DIGESTION SYSTEM

I. Specific Instructional Objective

After finishing this experiment hopefully students can evaluate the activity of antidiarrheal drug/pharmaceutical test.

II. Introduction

Diarrhea from *dia*: tought; *rhein*: flow, generally defined as increased the frequency of defecation and the abnormal of faeces form or liquid (Navaneethan and Ralph, 2011). Also said as the increasing the abnormalities liquid, frequency (>3 days), the heavy of feses (>200 g per day). Component of the fluid as the prime determines of the volume and consistency of the faeces commonly around 70-85%. Pure component from the faeces liquid describes the balance of input and output in lumen. The lumen input consist of ingestion and also water and electrolyte secretion meanwhile the output is the absorbtion a long the ingestion channel. The imbalance between input and output in lumen, will induced diarrhea. This balance is protected by digestion system with water, mineral, and nutrient extraction from lumen also remnant a number of liquid that appropriated to output trash substance easily by defecation.

At the normal condition, total absorption capacity from small intestine is 16L and colon is 4-5L. Neorohumoral mechanism, pathogen, drugs can change it in absorption or secretion, and also motility alteration (Sunoto dan Wiharta, 1987).

At the normal condition, the food contained in the stomach is digested become chymus and directly to intestine to make apart by the enzyme of digestion system. After all the nutrient was reabsorbed by villi entered to the blood, the residu of chymus that contain 90% water and the rest of metabolism that hard to be digested will passed to the colon. Furthermore, the flora normal will digested the residue, so it can be absorbed during the passage trough the colon. The water also being reabsorbed thus, sooner or later the contents from intestines will be solidify and become faeces when it was excretion from the body. The accumulation of fluid in the intestine due to disturption of water reabsorption or the hypersecretion (Tjay dan Rahardja, 2007).
Diarrhea can be caused by infection of bacteria, virus, drug, food, sweeteners, caffeine, alcohol, and also in Premenstrual Syndrome conditions. Based on the pathophysiology of diarrhea, it can be divided into osmotic diarrhea, secretory diarrhea, exsudative diarrhea, and motility. The continuous diarrhea must be checked as it can result in dehydration, loss of nutrient, and metabolic acidosis due to release of HCO$_3^-$ (Sherwood, 2011).

### III. Antidiarrheal Testing Methods

The activity of antidiarrheal is limited to the activity drug that can retard intestine peristalsis, thus decreased the frequency of defecation and repair the consistency of the faeces.

a. Transit Intestine method

   It used to evaluate the activity of antidiarrheal agents based on the influence to intestine distance ratio that adopted by a marker in a certain time toward to the overall length of the colon in animal studies in mice and rats.

b. Protection method of diarrhea by *Oleum ricini*

   Triglyceride from ricinoleic acid contained in *Oleum ricini* will be hydrolyzed in the intestine by enzyme lipase pancreas into glycerine and ricinoleic acid that can decrease fluid absorption and electrolytes and stimulates the peristalsis. The drugs efficacy in antidiarrheal will protect experimental animal against diarrhea that induced by *Oleum ricini* (KKIPM, 1993).

### IV. Antidiarrheal Drugs

Based on the pathogenesis of diarrhea and also the effect of pharmacology, medication of antidiarrheal divided in to 5 groups (Sunoto dan Wiharta, 1987):

a. Adsorbent drugs: Kaolin, Bismuth subsalicylate, the active carbon

b. Antisecretory drugs: Cholestyramine, Bismuth subsalicylate, Racecadotril

c. Antimotility drugs: Loperamide, Diphenoxylate, Octreotide, Racecadotril

d. Anticholinergic drugs: Belladonna alkaloids, Atropine, Hyoscyamine

e. Antimicrobial drugs: Tetracycline, Furazolidone, Chloramphenicol, Cotrimoxazole

In addition, is also required the provision of oral rehydration for patient in diarrhea to replace the fluid loss cause of diarrhea.
V. Method

5.1 Tools
Syringe with oral tube, rat *restrainer*, the surgery tool, ruler

5.2 Materials
*Oleum ricini*, Loperamide, chinese ink as a marker, plant extract, and suspension CMC 0,5%.

5.3 Animal
The animal used are rats with 150-200 g weight

5.4 Procedure
1. Divided rats into 3 groups:
   
   I : Control groups, give the chinese ink 1 ml
   II : *Oleum ricini* 2 ml and chinese ink 1 ml
   III : Extract
   IV : Suspension of Loperamide 0,05% doses 1,4 mg/kg BB

2. After 60 minute, gives *Oleum ricini* 2 ml.

3. At the minute 120 all the animal gives chinese ink 1 ml.

4. At the minute 180 all the animal sacrificed by cervical dislocation. Remove the intestine carefully, and measure the instestine that trough by the carbon active start from pylorus until the last part of intestine (black) and the entire length of the intestine from pylorus until ileocecal valve of each animal.

5. Calculate the percentage that passed by the marker ink by the whole length of the colon.

6. Analysis in statistic the percentage between test group and comparison group, and test group with control group.
VI. Report Experimental Data

Experiment title: 
Date of experiment: 
Group: 
Responder: 
Assistant supervisor: 

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<tr>
<th>Group</th>
<th>Rat</th>
<th>Overall length of intestine</th>
<th>The length of intestine that passed by a marker</th>
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<td>Average</td>
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<tr>
<td><em>Oleum ricini</em> + Chinese Ink</td>
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REFERENCES


EXPERIMENTAL GRAPH
DRUG EFFECT TO DIGESTION SYSTEM
CHAPTER 10. DRUGS/TESTED SUBSTANCES EFFECTS ON THE IMMUNE SYSTEM

I. Specific Instructional Objective
After finishing the experiment, the students are expected to be able to evaluate the effectiveness of an anti-allergy drug / tested substance with active cutaneous anaphylaxis method.

II. Introduction
Mast cells are inflammatory cells those are found on the tissue, originated from hematopoietic cell proliferation and differentiation of bone marrow. These cells have a role in responding to the signal of innate immunity as well as adaptive immunity by releasing inflammatory mediators, with an immediate or delay reaction. Mast cells were often found, especially in the bloodstream or in the tissues of body (Stone et al. 2009). The main mediators released by mast cells inside rejoin to the presence of noxious strange agents get in the body is called histamine. Aside its function to protect against incoming agents, histamine also has other effects, such as contraction of smooth muscle, endothelial cells, and the nerves points. Human mast cells containing approximately 2 to 5 pg histamine per cell (Prusin & Metclafe, 2003). Specific allergen T helper cells have an important role on the pathogenesis of hypersensitivity reactions. T helper cells activate the immune complex reaction (IgE-mast cell) that trigger the release of potent mediators and increasing the inflammatory cells (Nauta et al., 2008).

Anaphylaxis is an adverse allergic reaction that occurs rapidly and systematically, affecting one or more organs. Anaphylaxis reactions can occur after being exposed to foods containing protein, medications, insecticide and objects were allergen (Boyce et al. 2009). Anaphylaxis reaction is triggered by cross-linking between the Ig-E and aggregation FcR1 receptors on the surface of mast cells and basophils (Simons & Sampson, 2008). At the time of incoming antigen inside, mast cell will be activated (through Ig-E fragment which patched to the surface of mast cells), initiating histamine release from the mast cells (Kemp & Lokey, 2002). As the consequences, the amount of histamine released is too much and unable to be metabolized by the histaminases enzyme, this excess histamine will cause physiological disorders in tissues and organs, such as bronchoconstriction, blood vessels dilation, edema, contractions of the digestive tract (Leung & Ledford, 2009).
III. Screening Method

There are several methods that can be used to determine anti-allergy activity of a compound or therapeutic agents. Animal model which sensitized by antigen originated from proteins and antibodies outside, can be used as experimental animals in \textit{in vivo} studies. Injection of protein antigen into the body of the animal by subcutaneous/intradermal will stimulate active cutaneous anaphylactic reactions, injection of Evans blue solution after sensitization (7 to 14 days) will bring a blue lump on the sensitization area (Arimura et al. 1990). Injection of antibodies to the animal body by subcutaneous/intradermal would trigger a passive cutaneous anaphylaxis reaction, after a latency period (24 to 72 hours), repeated injection of antibodies with Evans blue causes the appearance of a blue lump on sensitization area (Park et al., 2005). The release of histamine from mast cells can also be determined by stability of mast cells by \textit{in vitro} study, the ability of a compound or therapeutic agents on inhibiting degranulation of mast cells can be observed microscopically (Guptha et al. 1995). The resulting histamine levels can also be determined by spectrofluorometry method (Shore et al. 1959). However, \textit{in vitro} study is rarely conducted in practical study scale, because the process and preparation of materials, sample cells and histamine are relatively complicated.

IV. Antihistamines (Anti-Allergic)

Based on its mechanism action, antihistamines can be divided into three groups, namely the histamine receptor antagonist, inhibitor release of histamine and anti-IgE (McKay and Oosterhout, 2000).

a. H1 histamine receptor antagonists

This drug works by occupying the histamine receptor, so that inhibiting the physiological effects of histamine. Examples: Dexchlorofeneramin, Diphenhydramine, Promethazine.

b. Inhibiting histamine release

This drug works by preventing the release of histamine from mast cells. The mechanism of action of this drug is to inhibit and decrease the Ca2 + influx into cells, and inhibit the activation of mast cells. Examples: Cromolyn sodium, Sodium cromoglycate and Nedocromil sodium.

c. anti-IgE

This drug is relatively new. This drug is a monoclonal antibody which act on IgE, which is responsible for activating mast cells to release histamine. Examples: a monoclonal antibody Omalizumab.
V. Method

5.1 Tools
Animal scale, 1 mL syringes, beaker glass 50 mL, Erlenmeyer 50 mL, stopwatch, shaver, ruler

5.2 Materials
Ovalbumin, NaCl 0.9%, Methylene blue, CTM 1%, CMC sodium

5.3 Animal
The animals used are rats

5.4 Procedure
1. One week before the experiment, the animals were weighed and marked.
2. Several animals were divided into groups.
3. Animal sensitization was conducted by injection of ovalbumin suspension in NaCl 0.9% 0.6 ml ip and 3 days later with ovalbumin suspension in 0.9% NaCl 0.1 ml intraplantar basis.
4. On practice, animals that have been sensitized, shaved the furs in dorsally then treated with CTM (1% 6 mg / kg) and the extract suspension at dose of 100 mg / kg.
5. After 1 hour, the animals were injected with methylene blue solution 0.2 mL through the tail vein.
6. Again, animals were injected with ovalbumin on the previous sensitized site subcutaneously.
7. Observations under intervals time of 30, 60 and 80 minutes.
8. Active cutaneous anaphylaxis characterized by the appearance of a blue lump on the injection area (dorsally).
9. Given a score on the observations according the following table.

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<th>Color intensity In Area</th>
<th>Score</th>
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<tbody>
<tr>
<td>Colorless</td>
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<td>No irritation</td>
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<tr>
<td>A little bit blue</td>
<td>2</td>
<td>Skin-deep</td>
</tr>
<tr>
<td>Bright blue</td>
<td>4</td>
<td>Skin-deep</td>
</tr>
<tr>
<td>Dark blue</td>
<td>6</td>
<td>Moderate (&gt; 4)</td>
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<tr>
<td>Dark blue lump</td>
<td>8</td>
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VI. Report Experimental Data

Experiment title :
Date of experiment :
Group :
Responder :
Assistant Supervisor :

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<td></td>
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<td>30 min</td>
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<tr>
<td>1</td>
<td>CMC 0,5 %</td>
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<tr>
<td>3</td>
<td>CTM</td>
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VII. Discussion
REFERENCES


GRAPHICS OF EXPERIMENT
DRUGS EFFECTS ON IMMUNE SYSTEM