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Original Article

THE IMPACT OF PEPTONE-INDUCED HYPERTHERMIA ON FETAL ANOMALIES IN WHITE MICE (*MUS MUSCULUS*)

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ABSTRACT

Objective: Hyperthermia is an increase in body temperature above 37.5 °C, which can be caused by environmental factors such as infections that disrupt hypothalamic thermoregulation and trigger a rise in body temperature.

Methods: The aim of this study was to assess the impact of hyperthermia on fetal anomalies in white mice using 10% peptone as the inducer. The study involved 15 white mice divided into three groups. Peptone 10% was administered to two treatment groups, with the administration time divided into two phases: group 1 on days 3-7 of mouse pregnancy and group 2 on days 8-12 of mouse pregnancy. Parameters observed included maternal weight gain, number of fetuses, and fetal weight analyzed using one-way Analysis of Variance (ANOVA) test, as well as morphological and skeletal abnormalities analyzed descriptively through photographs.

Results: The average initial temperature of the female mice in the control group, group 1 and group 2 was 35.4 °C, 35.7 °C, and 35.8 °C, respectively, while the average final temperature of the female mice in the control group, group 1 and group 2 was 35.4 °C, 38.0 °C, and 37.9 °C, respectively. The results showed no significant differences in maternal weight gain and number of fetuses between the treatment group and the control group ($p>0.05$). However, there was a significant difference in fetal weight between the treatment group and the control group ($p<0.05$). Descriptive observations revealed anomalies in fetuses fixed with Bouin's solution, including resorption sites, delayed growth, and hemorrhage. In contrast, fetuses fixed with alizarin red showed defects in the 14th rib, metacarpal, and metatarsal bones.

Conclusion: Based on these findings, it is concluded that hyperthermia can affect fetal anomalies in white mice.

Keywords: Hyperthermia, Peptone, Anomalies, Morphological, Skeletal

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INTRODUCTION

Teratogen is an agent that can cause abnormalities in fetal development during pregnancy. These fetal developmental congenital anomalies can include functional, structural, or genetic abnormalities [1]. Birth defects broadly defined as inborn developmental errors that affect physical, intellectual, and social well-being, have a significant impact on population health globally. A recent animal study identified a molecular mechanism for hyperthermia-induced teratogenicity, involving the temperature-activated ion channels these selectively bind to the nociceptive transient receptor potential vanilloid 1 (TRPV1) ion channel [1]. Transient receptor potential vanilloid 4 (TRPV4) in neural crest cells during the critical period of fetal development [2]. Congenital anomalies,known as birth defects or congenital disorders, are structural or functional abnormalities that occur during intrauterine life. They can be identified prenatally, at birth, or later in life. These anomalies contribute significantly to mortality rates in both developed and developing countries [3]. Hyperthermia is an increase in body temperature above 37.5 °C, which can be caused by environmental factors such as excessively high ambient temperature, infection, dehydration, medications, and changes in the central temperature regulation mechanism related to birth trauma to the brain [4]. Hyperthermia can cause membrane disruption, cell death, vascular disturbances, and placental infarction. It can also lead to severe fetal malformations or death. There were several impacted such as including the fetal stage of development, prior exposures and maternal immunity, individual immune variables, the placenta's ability to protect the fetus from infections, and the development of the fetal immune system [5].

There are 3 methods that can be used to test hyperthermia activity, namely fungal induction, lipopolysaccharide induction, and peptone induction. In this study, peptone was used because it is more non-toxic than other methods. Peptone is a derivative of protein resulting from hydrolysis, characterized by its solubility in water, resistance to coagulation when heated, resistance to salting out with ammonium sulfate, and the ability to precipitate when reacting with alkaline reagents such as phosphotungstic acid. Hydrolyzed peptones can be used as microbial growth media for a nitrogen source. Peptone is a type of protein and a pyrogenic compound that induces fever. Excessive protein consumption can alter the protein balance in the body and stimulate the release of cytokines that trigger the hypothalamus to increase prostaglandin production, altering the set point in the hypothalamus and causing hyperthermia [6-8]. Peptone will enter the body and be considered an antigen. Peptone contains lipopolysaccharide which is a component of the cell walls of g-negative bacteria which activates the immune system. Peptone will stimulate phagocytosis which causes the release of IL-1. IL-6, and IFN [9].

Hyperthermia caused anomalies such as exposure and maternal immunity, individual immune variability, the placenta's ability to protect the fetus from infection, and the development of the fetal immune system [10]. Further research on the teratological effects of peptone as an inducer of increased body temperature is still limited. Apart from that, considering the many effects that can occur if pregnant women experience hyperthermia, it is necessary to carry out research regarding the effects of hyperthermia. Therefore, this study investigates hyperthermia induced by 10% peptone orally on fetal abnormalities in mice.

MATERIALS AND METHODS

Materials

Test animals (Wistar female white mice (*Mus musculus*) are about 2 mo old and the white male of the wistar strain is around 3 mo old and weighs 20-30 g 15 of them are in good health), peptone, distilled water, Bouin's solution, and alizarin red.

Preparation of experimenta animals

Ethical approval for the test animals was obtained from the Faculty of Pharmacy Ethics Committee of Universitas Andalas, with approval number 13/UN.15.10. D. KEPK-FF/2024 signed on January 30. 2024.

Mice with a regular estrous cycle of 4-5 d were used. They had a body weight between 20-30 g and were placed in maintenance and treatment cages. They were acclimatized for 10 d to observe the estrous cycle by visually inspecting the vaginal condition of the mice. Mice in estrus showed specific signs such as a redder color and increased moisture. Acclimatization is the process of maintaining laboratory animals with the aim of adapting them to a new environment, so that when procedures such as surgery or other actions are performed, it is expected that the animals have reduced stress levels due to the change from their previous environment [11, 12].

Healthy animals were those that did not experience more than a 10% fluctuation in body weight and had an estrous cycle ranging between 4-5 d. During estrus, female mice were mated with males at a ratio of 1:4 [13]. In the morning, vaginal plugs were checked. The presence of a vaginal plug indicated that the mice had copulated, and this day was considered day 0 of pregnancy. Pregnant mice were separated from those not yet mated, while the unmated ones were reintroduced to the male mice.

Preparation of 10% peptone solution

Peptone weighing 1 g was placed into a beaker and dissolved in 10 ml of distilled water while stirring.

Treatment of experimental animals

The experimental animals were divided into 3 groups, with each group consisting of 5 mice. It is provide for an experiment is determined by a number of factors, including the desired power of the experiment, the effect size, the population standard deviation, and the significance level. The rectal temperature of the mice was measured using a digital thermometer. To avoid stress, mice were placed in a quiet place before checking their body temperature. In addition, to cage correction and enrichment, gentle handling and training should be used as fine-tuning protocols before experimental procedures are performed. Animal training or structured human-animal interaction reduces stress in laboratory animals and has been effective for many species of laboratory animals [21]. The goal of mice to avoid stress is so that there is no weight loss caused by stress [23]. Mice were induced with hyperthermia using 10% peptone orally, for 30 min and 60 min, and their rectal temperature was then remeasured using a digital thermometer. The temperature was measured before 10% peptone has been give and after 30 min and 60 min after giving the peptone orally [9].

Table 1: Experimental animal groups

Control group	Aquadest
Group I	Peptone 10% on d 3-7 of pregnancy
Group II	Peptone 10% on d 8-12 of pregnancy

Data analysis

The data on mother mouse weight, number of fetuses, and fetus weight will be tested using statistical analysis using Microsoft Excel with the formula (=STDEV. S: To find the SD of the sample) and (=AVERAGE: to find the average weight of the sample). The observations obtained will be processed using SPSS software with ANOVA test. The test was carried out to see the relationship between peptone administration and an increase in mice body temperature and fetal body weight. Observations on defect types and fixation with Bouin's solution and Alizarin Red will be analyzed descriptively in control groups.

RESULTS

Peptone-induced affects hyperthermia of mice

After statistical testing using one-way ANOVA, a significant increase was observed in the treated groups, specifically group 1 and group 2 compared to the control group ($p<0.05$). The average final body temperatures of the mother mice in the control group, group 1 and group 2 were 35.4 °C, 38.0 °C, and 37.9 °C, respectively. This demonstrates that 10% peptone can induce hyperthermia in mice.

Peptone-induced hyperthermia affects the fetal weight of mice

The observation results showed that the control group had fetus weights within the normal range, which is 0.7-1.2 g. However, those given 10% peptone on days 3-7 of pregnancy and days 8-12 of pregnancy had significantly lower fetus weights. Statistical testing results showed a significant difference between group 1 and group 2 in terms of fetus weight ($p<0.05$). The one-way ANOVA gave result that *p values* of maternal weight gain, fetal weight, temperature differences was 0.103. 0.360. and 0.000. The value illustrates that there is a significant difference between the negative control and the sample.

Table 2: Mean±SD increase in mice temperature after 30 min and 60 min of preparation administration

Time	Temperature (°C elcius)		
	Control group	Group 1	Group 2
30 min	-0.12±0.08	1.03±0.26	0.96±0.13
60 min	-0.03±0.07	1.26±0.27	1.12±0.22

Table 3: Average fetal weight of mice

No.	Increase of weight (g)		
	Control group	Group 1	Group 2
1	0.97	0.74	0.87
2	1.05	0.67	0.70
3	0.90	0.48	0.48
4	0.78	0.92	0.79
5	1.00	0.70	0.67
$\bar{x}$±SD	0.94±0.10	0.70±0.14	0.70±0.13

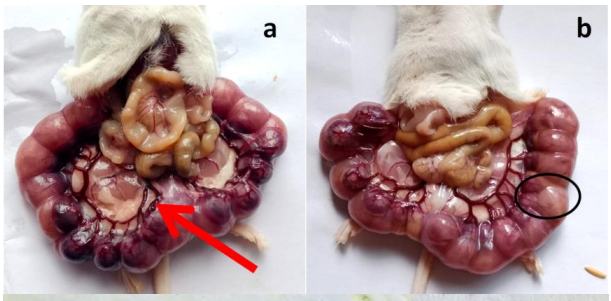
$\bar{x}$: Average of mice weight, SD: Standard Deviation

Hyperthermia can cause fetal anomalies in white mice

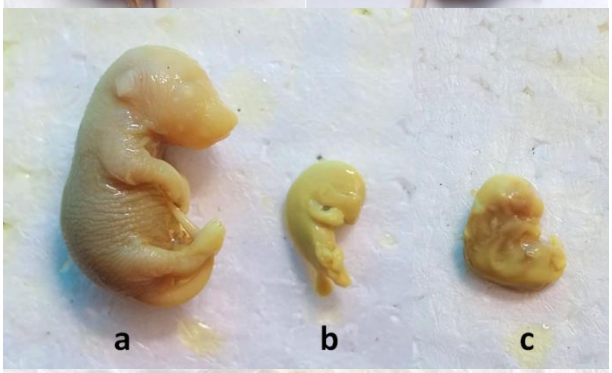
Hyperthermia can cause hypertrophy, resorption sites, and delayed growth. Hemorrhage was found in the head, limbs, abdomen, and tail of the foetuses and The results of the skeletal observations showed incomplete development in the metacarpal and metatarsal bones, marked by a reduced number of ossification segments, and the presence of rudimentary or incomplete 14th ribs. It is 1 of 5 mice on group 2 has lower ribs. The normal ribs should be 28, one of it has 27 ribs in group 2, there were 3 mice that did not have metacarpals and metatarsals. The most significant development was in the metacarpals, where all 5 mice had abnormalities in the metacarpals when administered peptone 10% on days 8-12 of pregnancy.

Table 4: Anomalies observation figures

a The arrow indicates hypertrophy of the uterus could interfere with blood flow and nutrients necessary for fetal growth
b The circle indicates resorption site.

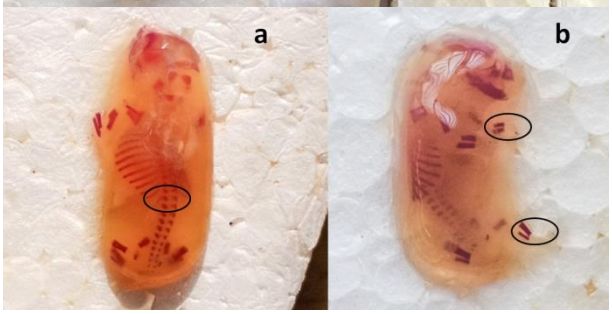


a Normal fetus
b Fetus with delayed growth
c Resorption site after fixation with Bouin's. It is showed malformation and delayed growth.



The circles indicated fetus with haemorrhage that could interfere the brain development

a The circles indicated rudimentary rib not paired
b The circles indicated incomplete metacarpal and metatarsal bones



DISCUSSSION

Teratogenicity, or the ability to cause birth defects, can arise from various sources. These include environmental factors like drug use, exposure to radiation, infections, and deficiencies in hormones or vitamins, as well as genetic factors such as chromosomal abnormalities and mutations. Numerous substances and conditions can induce teratogenic effects in humans or animals, including ionizing radiation, infections from cytomegalovirus, rubella virus, herpes virus, and syphilis, as well as metabolic imbalances caused by alcohol consumption during pregnancy and folic acid deficiency [14]. The test preparation was administered on days 3-7 of pregnancy for group 1 and days 8-12 of pregnancy for group 2. During this period, organogenesis occurs in mice, a phase where cells undergo intense proliferation through mobilization, differentiation, and organization processes. Therefore, hyperthermia resulting from the administration of peptone can inhibit and damage developing cells at this stage. The body temperature of the mice was measured rectally before administration of the preparation, and 30 min and 60 min after administration using a digital thermometer. The initial average body temperature of the mother mice in the control group, group 1 and group 2 was 35.4 °C, 35.7 °C, and 35.8 °C. Statistical analysis using one-way ANOVA showed a significant increase in the treated groups, group 1 and group 2 compared to the control group ($p<0.05$) with the final average body temperature of the mother mice in the control group, group 1 and group 2 being 35.4 °C, 38.0 °C, and 37.9 °C. This demonstrates that 10% peptone can cause hyperthermia in mice. Peptone itself is pyrogenic, which can increase body temperature in experimental animals (table 2). In another study, it was found that a significant increase in body temperature occurred in mice given peptone at 30. 60. 90 and 120 min with p value = 0.017 [9].

The observation results showed that the control group had fetus weights within the normal range, which is 0.7-1.2 g. However, those given 10% peptone on days 3-7 of pregnancy and days 8-12 of pregnancy had significantly lower fetus weights. Statistical testing results showed a significant difference between group 1 and group 2

in terms of fetus weight. This difference is intriguing and warrants further investigation. It is likely due to the different timing of peptone administration during the mice's pregnancy. Administering peptone to group 1 on days 3-7 of pregnancy might have a different impact compared to administering peptone to group 2 on days 8-12 of pregnancy. This could be because the early stages of fetal development are crucial and sensitive to environmental influences and substances consumed by the mother. Additionally, the difference in the range of fetal weights between the two groups is also noteworthy. The wider range in group 1 might indicate greater variation in response to peptone administration during the early stages of pregnancy.

Table 5: The average weight gain of the mother during pregnancy, the number of fetuses, and the weight of the mouse fetus

Research parameters	Average±SD		
	Control group	Group 1	Gruup 2
Mother's weight (gram)	22.13±4.02	20.76±1.89	20.76±1.89
Number of fetuses	10.80±2.93	12.60±1.62	13.00±1.90
Weight of fetuses (gram)	0.94±0.10	0.70±0.14	0.71±0.13

SD: Standard Deviation

Morphology serves as an observation parameter to identify any external abnormalities in the fetus, including the completeness of the hands and legs, the presence of hemorrhage, body deformities, and dwarfism. These observations are conducted macroscopically and are crucial for teratogenic testing. In the visual observation of mouse fetuses, two types of fixation are employed. Fixation involves immersing the fetus in fixative solutions, namely Bouin's solution and alizarin red solution. The primary goal of fixation is to preserve cells and tissue components in a "life-like state." Fixation aims to prevent or arrest the degenerative processes that begin immediately after tissues lose their blood supply. Bouin's solution is used in these experiments to examine the morphological state of the fetus, as it hardens the tissue and imparts a yellow color [15].

On day 18 of pregnancy, the mice underwent laparotomy. Laparotomy is a surgical procedure to remove mouse fetuses by making an incision in the pregnant mouse's abdominal area. Before laparotomy, the pregnant mouse is euthanized by cervical dislocation, achieved by pressing on the mouse's neck with a blunt object and then pulling its tail firmly. This causes instantaneous death in the pregnant mouse. Observations are then made on uterine hypertrophy, implantation sites, and fetuses showing growth retardation during the laparotomy process. .After the laparotomy procedure, observations were made regarding the number of fetuses and their weight There were eight dead fetuses in group 1 and four dead fetuses in group 2. The deaths of the fetuses are suspected to be due to genetic vulnerability factors caused by administered preparation. In group 1. The preparation was given on days 3-7 of pregnancy, during which there is a high likelihood of fetal death. Dead fetuses were characterized by the absence of movement when removed from the uterus. Additionally, the differences in the range of fetal weights between the two groups are also noteworthy.

Hypertrophy of the mouse uterus, causing abnormal enlargement of blood vessels, can disrupt the flow of blood and nutrients needed for fetal growth. This disruption can lead to resorption sites, where the placenta loses its ability to provide adequate nutrition to the fetus, causing growth delays. This condition indicates a serious disturbance in the uterine environment that can affect the health and development of the fetus. Another abnormality is hemorrhage, which occurs when blood leaks from the cardiovascular system and accumulates in body tissues due to differences in fluid viscosity pressure between plasma and capillaries. The cause of hemorrhage is vasoconstriction, which creates high blood pressure and can lead to vessel rupture (bleeding) [15]. This vasoconstriction is triggered by the contraction of smooth muscles in the uterus caused by the administration of 10% peptone. Peptone is an exogenous pyrogen compound that can stimulate cells in the immune system to synthesize cytokines. Cytokines then induce the production of prostaglandin E2 (PGE2), which causes peripheral vasoconstriction, ultimately leading to hyperthermia. Hyperthermia interferes with protein synthesis via heat-shock proteins, which can entail membrane disruption, cell death, vascular disruption, and placental infarction. This can induce severe fetal malformations or death [10].

To observe ossification, the fetuses were first immersed in a red alizarin solution, which stains the bones for easier examination. However, using alizarin solution can be challenging because the specimens are very delicate and can be easily damaged when handled or transferred to other observation areas. Observing skeletal abnormalities with this method requires high precision and care, as any damage from external impacts or during specimen transfer may be mistaken for defects, leading to inaccurate results [18]. Resorption site is an embryo that does not receive nutrients due to the inhibition of blood flow to the embryo or fetus caused by hypertrophy. Sufficient blood flow to the reproductive area is crucial to support embryo growth and development. If blood flow to the embryo is obstructed, it can lead to insufficient supply of nutrients and oxygen needed for normal growth. This can result in serious growth and developmental disorders, and as a response to conditions unsuitable for normal survival, resorption sites can occur. The introduction of foreign bodies into the body can indeed disrupt the normal physiological balance, affecting various aspects of osmosis, fluid pressure, and viscosity within the embryo. These disturbances can lead to significant issues such as changes in the pressure and viscosity of fluids between different compartments, including blood plasma, extra-capillary spaces, and intra-and extra-embryonic fluids. Such disruptions can result in serious consequences such as the rupture of blood vessels and subsequent hemorrhaging. This underscores the delicate balance and sensitivity of embryonic development to environmental influences and the potential risks associated with foreign body introduction during this critical phase. Some studies carried out using mice have looked at the incidence of cranio-facial defects following exposure on days 8–9, which includes the susceptible stage for neural tube closure. The formation of the eye, face, heart and vertebrae also take place at this time. Other studies have examined effects on brain and behaviour following exposure on days 12–15. A period of neuronal proliferation, migration and differentiation in the mouse brain [21].

Next, observations were conducted on skeletal development using alizarin red solution. The observations covered general skeletal growth, starting from the skull to the tailbone or caudal skeleton. Abnormalities in position, number, or shape different from those in the control animals can be considered as defects. These observations are challenging because specimens in alizarin red solution are highly susceptible to hardening when transferred to the observation area. Ossification is the process of bone formation that occurs during both prenatal and postnatal development. This ossification process takes place between days 11 and 15 of pregnancy. Bone staining is an important method for monitoring bone formation (ossification) developmentally, from early stages to completion [20]. Fetal bone staining is an integral part of embryological studies and the developmental structure of animals.

The results of skeletal observations showed incompleteness in the metacarpal and metatarsal bones, marked by a decrease in the number of ossification segments, as well as the presence of rudimentary or incomplete 14th ribs. Graham (2020) experiments showed there are strong associations between neural tube defects and maternal fever, and other studies have demonstrated

associations between first trimester hyperthermia and an increased risk for cardiovascular defects, oral clefts, isolated congenital ear defects, cataracts, hypospadias, renal anomalies, possibly anorectal malformations, and congenital anomalies in general, suggesting that this association between maternal hyperthermia and birth defects in humans is causal [22]. Not all fetuses within a group exhibited the same morphological abnormalities due to genetic variation among individuals, even though they originated from the same mother. Some fetuses may not show any morphological abnormalities at all, while others may experience one or even more abnormalities. The anomalies observed in the fetuses were suspected to be caused by hyperthermia resulting from the administration of 10% peptone. Hyperthermia can inhibit blood flow to the placenta, obstructing the intake of nutrients needed by the fetus. This is due to a decrease in the oxygen available for the metabolism of developing organs, including the minerals needed for bone growth, such as during the processes of ossification or calcification. During organogenesis, the embryonic bone framework transforms into bone tissue through primary ossification, followed by bone growth and calcification as it ages[4]. In future research, a higher dose of peptone can be used higher to increase the temperature during pregnancy. Dosage variations are also needed to see how differences in body temperature can cause abnormalities. Apart from that, it is necessary to observe various other indicators to determine fetal defects in mice. Conducting dose-response would be necessary for experiments to evaluate the effect of varying peptone concentrations on hyperthermia and anomalies.

CONCLUSION

Peptone with a concentration of 10% Inducing mice with a gestation period of 3-12 d produces effects in the form of hyperthermia, hypertrophy, hemorrhage, and site resorption. This hyperthermia causes skeletal abnormalities in mice. Abnormalities occur in the metacarpal, metatarsal and rudimentary bones rib or incomplete 14th rib. Therefore, it is concluded that giving 10% peptone to induce hyperthermia during pregnancy has more effect mouse fetal anomalies.

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AUTHORS CONTRIBUTIONS

All authors have contributed equally

CONFLICT OF INTERESTS

The authors declare no conflict of interest

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