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Research Article
Toxicity of Patiwala Leaf Extract (*Lantana camara* Linn.) as Antifertility Against Pregnancy in Rat (*Mus musculus* L.) Preimplantation Stage

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Abstract

Background and Objective: Expert biological pest control is thought to be safer and to have fewer side effects. The use of plants carrying antifertility compounds is one method that can be developed to biologically reduce rat pest populations and thwart reproduction. This study aims to determine whether the toxicity of patiwala leaf extract (*Lantana camara* Linn.) can interfere with preimplantation stage rat (*Mus musculus* L.) pregnancy. **Materials and Methods:** This study was an experimental study arranged in a completely randomized design (CRD) consisting of 4 groups with 6 replications namely control (no treatment), patiwala leaf extract concentrations of 2 g/kg b.wt. (P1), 4 g/kg b.wt. (P2) and 6 g/kg b.wt. (P3). Mice were treated orally by gavage using a 1 mL disposable syringe on the 1st day of pregnancy (preimplantation) for three consecutive days. Mice were dissected at 16 days of gestation, observations were made of the percentage of implantation (%IM), the percentage of gestational loss (%KGE) and the percentage of postimplantation mortality (%KPI). Data were analyzed by Analysis of Variance (ANOVA) using KaleidaGraph 95% confidence level and Tukey's HSD test. **Results:** The toxicity of patiwala leaf extract significantly interfered with the preimplantation stage of pregnancy in rats. The P3 treatment (6 g/kg b.wt.) gave a higher toxicity effect, namely, the percentage of implantation success was only 25.67%, the percentage increase in gestational loss was 74.26% and the percentage of post-implantation death was 66.67%. **Conclusion:** The patiwala leaf extract at a concentration of 6 g/kg b.wt., interfered with the pregnancy of preimplantation stage rats because it could reduce the number of live fetuses.

Key words: Antifertility, *Lantana camara*, *Mus musculus*, preimplantation, toxicity

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Mice are a pest that is difficult to control because they can react or respond to control measures taken by humans[1]. Various methods have been used to control this pest population, but none have been truly effective[2]. The method of controlling the rat population chemically is considered quite dangerous because it can leave residues in nature that can result in the death of non-target animals or contamination of soil and water[3]. The control method being developed is biological control of the rat population[4]. Biological pest control by experts is considered safer and has fewer side effects[5].

One strategy that can be developed to biologically decrease rat pest populations and disrupt reproduction is the use of plants that contain antifertility chemicals. The activity of antifertility compounds is for contraception, abortion and wiretapping[6]. Compounds that are contraceptives function to prevent ovulation and fertilization[7], abortive causes the release of the fetus before implantation occurs and an interception, which works after fertilization occurs and disrupts the journey to implantation[8].

Research that aims to reduce fertility can be carried out on female rats because their reproductive tract has many areas that can be disrupted and their reproductive cycle was easier to disrupt or manipulate[9], whereas in male rats by reducing sperm quality[10]. Giving active ingredients to female animals can interfere with the process of ovulation, egg transport, zygote transport and implantation, reduce the number of offspring, inhibit lactation[8] and affect the behavior of the mother causing a decrease in the number of living children or inhibiting neonatal growth[11]. These disorders generally occur due to manipulation or interference given to the hypothalamic-pituitary-gonadal axis[12], so that the ratio and cycle of hormones in the three organs are disrupted which will directly or indirectly affect the reproductive process in test animals[13].

Antifertility compounds in plants generally belong to the group of steroids, alkaloids, isoflavonoids, triterpenoids and xanthones[14]. Several compounds found in *L. camara* leaves are classified as antifertility compounds, so this plant has the potential to reduce the fertility of rats[15]. The group of antifertility compounds is efficacious as a fertility regulator that is antigonadotropin. Interferes with oogenesis, prevents ovulation, blocks the meeting of the ovum with spermatozoa[12] inhibits implantation, increases the percentage of gestational loss and reduces the number of children[16].

Patiwala is the most dangerous weed in the world because it can cause poisoning and death in livestock[15]. Based on 1HNMR and TLC analysis on *L. camara*, it showed the presence of terpenoids in the nonpolar phase causing hepatotoxicity, heart and lung damage in rats[17], despite its utilization as a stomach ulcer drug[18], but the active compounds in *L. camara* leaves include saponins, phenols, flavonoids and essential oils[19], alkaloids, tannins and lantadine are toxic[3]. Patiwala leaf toxicity research on rats and livestock includes, Administration of polar and nonpolar extracts with concentrations of 1.5, 3.0 and 5.0 g/kg b.wt., causes acute toxicity in the form of hepatosis, tightness in the lungs and disturbances in the central nervous system[17]. The concentration of 2 g/kg b.wt., of *Lantana camara* leaves orally causes acute toxicity to the heart and kidneys of mice[20].

Research studies on the use of this plant as an antifertility in female animals to reduce the number of live births, so far have not been widely studied, so information about these studies is still very much needed. In this study, the toxicity test of patiwala leaves (*L. camara* Linn) was tested on preimplantation stage female rats (*Mus musculus* L.). The results of this study were expected that the leaves of this plant can be developed for antifertility materials in controlling rat pest populations or can also help the success of government programs that launch family planning programs.

MATERIALS AND METHODS

This research was carried out for two months from June to August 2022, at the Biology Laboratory, Faculty of Mathematics and Natural Sciences, Halu Oleo University, Kendari, Southeast Sulawesi Province, Indonesia.

Materials: The materials used in this study include, 24-female rats (*Mus musculus* L.) Swiss Webster strain obtained from mouse breeders in Kambu Village, Kendari City, Southeast Sulawesi, Indonesia with a weight of 29-34 g, 96% ethanol, patiwala leaf extract, rat feed in the form of commercial pellets (BP11-BRAVO) produced by PT. Charoen Pokphand Indonesia, chloroform, 0.9% NaCl, 10% ammonium sulfate, 10% potassium ferricyanide solution and 0.5% Na CMC.

Procedure: Extraction was carried out using the maceration method[21]. The dose of patiwala leaf extract was adjusted to the body weight of the rats with the formula: Extract dose = rat's body weight (g/kg b.wt.). The x dose (g)[22]. The dosage of patiwala leaf extract used was 2 g/kg b.wt. (P1), 4 g/kg b.wt. (P2) and 6 g/kg b.wt. (P3). Twenty-four female rats aged 12 weeks who were in the late proestrus phase were reared with adult male rats with a male: Female

ratio = 1:2 in the afternoon at 17.00 and the next day at 07.00 in the morning, vaginal smears were made. If there is a vaginal plug, pregnancy is declared on the 1st day and then the rats are given the extract treatment according to the treatment group once orally by gavage. The mice were then kept until they were 16 days pregnant and sacrificed by anesthetizing them using chloroform, then the mice were dissected for observation.

Observation: The number of implantations was obtained by counting all implantation sites, whether containing live fetuses, dead fetuses, or absorbed embryos along the two uterine horns. The parameters of the preimplantation stage of pregnancy observed in this method were the percentage of implantation (IM%), the percentage of gestational loss (KGE%) and the percentage of postimplantation mortality (KPI%).

Observation of the preimplantation stage of pregnancy parameters is calculated by the following formula[23]:

Implantation percentage (IM%):

$$\text{IM (\%)} = \frac{\text{Number of implantations}}{\text{Number of corpus leuteum}} \times 100$$

Percentage loss of gestation (KGE%):

$$\text{KGE (\%)} = \frac{\text{Corpus leuteum} - \text{number of implants}}{\text{Number of corpus leuteum}} \times 100$$

Post implantation death percentage (KPI%):

$$\text{KPI (\%)} = \frac{\text{Number of implantations} - \text{number of live fetuses}}{\text{Number of implantations}} \times 100$$

Statistical analysis: Data were analyzed by Analysis of Variance (ANOVA) using the KaleidaGraph with a 95% confidence level followed by Tukey's HSD test to determine the level of significance between treatments.

Ethical consideration: The ethical clearance was obtained from the Faculty of Natural Sciences and Mathematics with Letter Number UN.29.17.5/SK/EC/2022.

RESULTS AND DISCUSSION

Implantation percentage (IM%): Based on the ANOVA test and the Turkey HSD follow-up test at the 95% level, it was found that the average decrease in the percentage of implantation was significant with an increase in the concentration of the extract given. The percentage of rat implantation between treatment groups was presented in Table 1.

Based on the results of the ANOVA test ($p<0.05$) in Table 1 the average percentage of implantation for all extract treatments had a significant effect on the control group and the percentage value was below 50%. The treatment with a concentration of 2 g/kg b.wt. (P1) averaged a percentage of implantation of 49.04% and the treatment with a concentration of 4 g/kg b.wt. (P2) had an average percentage of implantation of 41.11%, while the treatment with an extract concentration of 6 g/kg b.wt., caused the greatest decrease in implantation that is equal to 25.67%. This was in line with research[24], that at high doses it causes an antifertility effect on pregnancy due to the entry of chemical compounds into the mother's blood vessels so that embryos at the cleavage stage are not able to develop to the blastocyst stage perfectly and cause the embryos to be unable to implant.

The antifertility effect on decreasing the percentage of implantation was due to flavonoids, saponin and triterpenoid compounds in patiwala leaf extract. This compound is antiestrogenic which plays a role in influencing ongoing implantation. Antiestrogenic compounds can cause the ovaries to become inactive and interfere with endogenous estrogen secretion[25]. Flavonoid compounds play a role in repairing granulosa cells in the ovary so that they can inhibit FSH and LH secretion. Obstacles to FSH and LH secretion will also inhibit the formation of progesterone and estrogen so that the uterine wall will shed and interfere with the implantation process[26]. Giving active ingredients to female animals can interfere with the process of ovulation and implantation and reduce the number of living children. Implantation failure is also caused by the uterus not being ready to receive the embryo at the time it should implant[27].

Patiwala leaf extract works effectively at the stage before implantation because it can survive in the body for quite a long time. Compounds from patiwala leaf extract are estrogenic[15], so they can bind to receptors in the cytosol and survive in the body for a long time. This was to the statement of Hirota[28], that the administration of estrogen at one day of gestation in mice keeps the eggs in the oviducts until 4 days of gestation. The average decrease in the percentage of implantation obtained for all treatments was below 50%. Alkaloid compounds in patiwala leaves have antiproliferative, embryotoxic and teratogenic properties, causing a significant decrease in implantation. This was by Sudiman *et al.*[29], which said that the cause of decreased implantation in mice was

Table 1: Percentage of implantation of the control and treatment group rats that were given patiwala leaf extract (*Lantana camara* Linn.) at one day of gestation

Group	N	$\bar{X}$ number of CL±SD	$\bar{X}$ number of IM±SD	$\bar{X}$ % IM±SD
Control	6	7.17±2.14	6.67±1.51	94.58±8.48^{a}
P1	6	7±1.79	3.5±1.87	49.04±22.37^{b}
P2	6	6.67±2.34	3.17±2.86	41.11±33.07^{bc}
P3	6	8.17±1.83	2.5±2.81	25.67±28.58^{def}

Numbers followed by the same letters in the same column show results that are not significantly different based on Turkey's HSD at a 95% confidence level, N: Repeat, CL: Corpus luteum, SD: Standard deviation, IM: Implantation and standard deviation (±)

Table 2: Average percentage of gestational loss in the control and treatment group rats that were given patiwala leaf extract (*Lantana camara* Linn.) at one day of gestation

Group	N	$\bar{X}$ Number of KL±SD	$\bar{X}$ Total IM±SD	$\bar{X}$ % KGE±SD
Control	6	7.17±2.14	6.67±1.51	5.41±8.47^{a}
P1	6	7±1.79	3.5±1.87	50.96±22.37^{b}
P2	6	6.67±2.34	3.17±2.86	58.8±33.07^{bc}
P3	6	8.17±1.83	2.5±2.81	74.26±28.63^{bcd}

Numbers followed by the same letter in the same column show results that are not significantly different based on Turkey's HSD at a 95% confidence level and standard deviation (±)

due to the disruption of the growth of the inner cells in the blastocyst wall, which form embryo cells and the outer cells will implant in the uterine wall, which forms the placenta. A substance can be categorized as an anti-implantation agent if it can inhibit implantation by 50%[30].

Percentage of gestational loss (KGE%): Based on the results of the study, it was found that the average percentage of gestational loss due to *L. camara* leaf extract gave significantly increased compared to the control group. Based on Turkey's HSD follow-up test at a 95% confidence level, of the three doses of patiwala leaf extract given, the toxicity that most significantly increased the percentage of gestational loss was the concentration of 6 g/kg b.wt. The results of observations of the average percentage of gestational loss between treatment groups were presented in Table 2.

The toxicity of patiwala leaf extract increases the percentage of gestational loss as the concentration of the extract is increased. In the 2 g/kg b.wt. (P1) extract treatment, the percentage increase in gestational loss was 50.96% and significantly different from the control. Treatment of the 4g/kg b.wt., extract increased the percentage of gestational loss by 58.8%, significant compared to the control but not too significant compared to the P1 group based on Turkey's HSD follow-up test at a 95% confidence level. The highest toxicity of *L. camara* leaf extract increased the percentage of gestational loss at a concentration of 6 g/kg b.wt. (P3). Based on the results obtained from the three treatments, showed that the percentage of gestational loss increased with increasing concentrations, this was due to the increasing number of antifertility compounds contained in *L. camara* leaves absorbed by the mother's blood. The triterpenoid and flavonoid compounds[31] in the leaf extract of *L. camara* interfered with the proliferative process of embryonic cells at the cleavage stage of embryogenesis, so they did not reach the blastocyst stage completely and as a result, the embryo could not be implanted[32].

The toxicity of *L. camara* leaf extract also causes embryo resorption and even fetal death. Based on observations, the toxicity of *L. camara* leaf extract caused a reduction in the number of live fetuses. Fetal death can be caused by hormonal imbalances during pregnancy. This was to the statement of Zaman *et al.*[33], that the factors that cause the fetus to survive in the uterus until before delivery are hormonal balance, availability of nutrients, temperature and metabolism of the mother's body. Fetal death is caused by the entry of teratogenic substances from patiwala leaf extracts such as flavonoids and saponins into the mother's body through the placenta so that the fetus cannot survive. According to Animaw *et al.*[34] that the entry of teratogenic compounds through the placenta and on the embryo in the pre-differentiation phase in most embryo cells causes damage and ends in the death of the embryo[15]. Teratogenic effects that reach the embryo can cause embryonic death followed by resorption at the beginning of pregnancy and fetal death at the end of pregnancy[35].

Percentage of post implantation deaths (KPI%): Based on the ANOVA test and Turkey's HSD follow-up test at a 95% confidence level, it was found that the toxicity of *L. camara* leaf extract at a concentration of 4 g/kg b.wt. (P2) and treatment with an extract concentration of 6 g/kg b.wt. (P3) significantly increased the percentage of postimplantation deaths compared to the control group. Extract treatment with a concentration of 2 g/kg b.wt., showed no significant toxicity compared to the control group. The

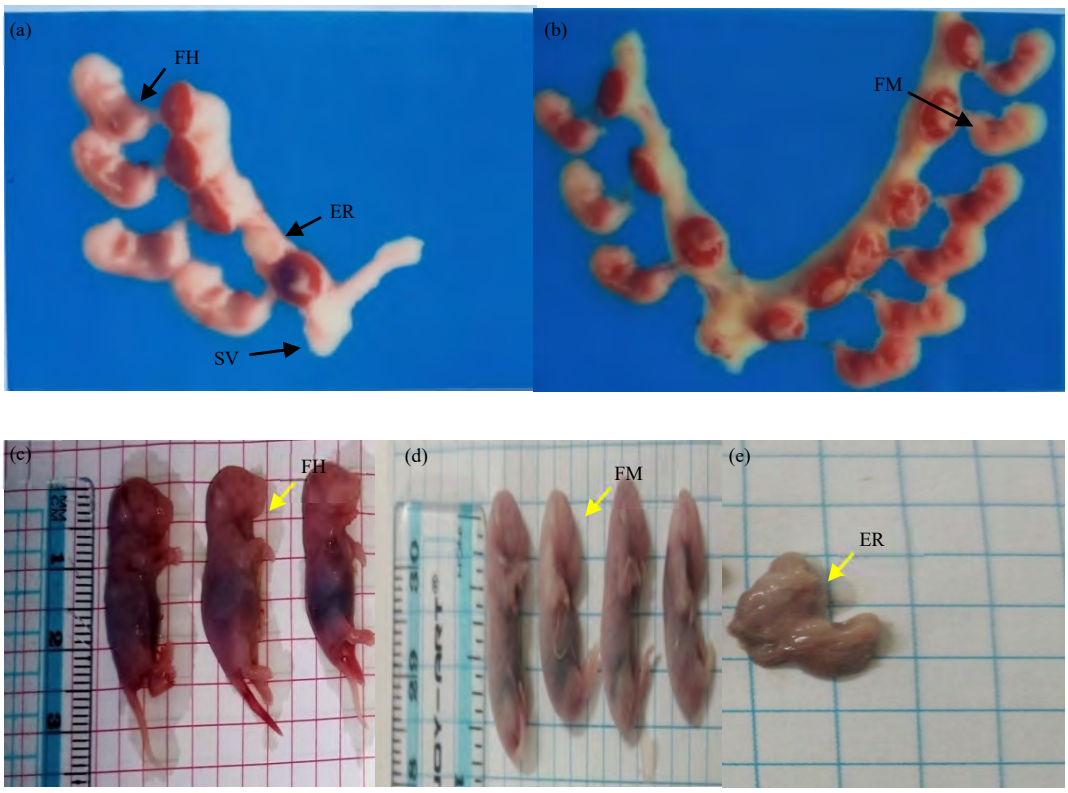

Fig. 1(a-e): Condition of the rat uterus due to the toxicity of patiwala leaf extract (*Lantana camara* L.), (a) Uterine horns with partially live fetuses (FH) and embryo resorption (ER) in the form of black spots showing traces of implantation, (b) Uterine horn with a partially dead fetus (FM), (c) Live fetus (FH) which is bright red in color and has a beating heart and blood vessels after being removed from the amniotic sac, (d) A dead fetus (FM) which looks pale and does not respond to touch and (e) Appearance of a resorption embryo (ER) in the form of remaining macerated embryo tissue

Table 3: Average postimplantation death percentage of control and treatment group rats that were given patiwala leaf extract (*Lantana camara* Linn.) at one day of gestation

Group	N	∑ IM $\bar{X}$ ±SD	∑ER $\bar{X}$ ±SD	∑FH $\bar{X}$ ± SD	% KPI $\bar{X}$ ±SD
Control	6	6.67±1.51	0±0	6.67±1.51	0±0^{a}
P1	6	3.5±1.87	0.17±0.41	3±1.55	11.11±17.21^{a}
P2	6	3.17±2.86	0.67±1.03	2.17±1.94	51.11±41.72^{ab}
P3	6	2.5±2.81	1.17±1.33	1±1.26	66.67±40.82^{bcd}

Numbers followed by the same letter in the same column show results that are not significantly different based on Turkey's HSD at a 95% confidence level, IM: Implantation, ER: Embryo resorption, FH: Live fetus, KPI: Postimplantation death and standard deviation (±)

highest percentage of post-implantation mortality was in the P3 group, which was 66.67%. The average percentage of post-implantation deaths between groups was presented in Table 3.

In this study, the high value of postimplantation mortality was caused by embryo resorption and fetal death. In rats treated with P2 at a dose of 4 g/kg b.wt., out of 3.17 implanted embryos, the average surviving fetus was 2.17 and 0.67 embryos were absorbed. In the P3 treatment at a dose of 6 g/kg b.wt., out of an average of 2.5 implanted embryos, only 1 fetus survived and 1.17 embryos were absorbed. The active compound in patiwala leaves is teratogenic so it affects the embryo in the pre-differentiation phase, as a result, most of the embryonic cells become damaged and end in the death of the embryo. Post-implantation death occurs when a new embryo is implanted and implantation failure often occurs due to failure of egg cell transport[36]. The state of the rat uterus with the state of the fetus after administration of patiwala leaf extract in one day's pregnancy was presented in Fig. 1.

The toxicity of patiwala leaf extract in the P3 group (6 g/kg b.wt.) also caused fetal malformations in the form

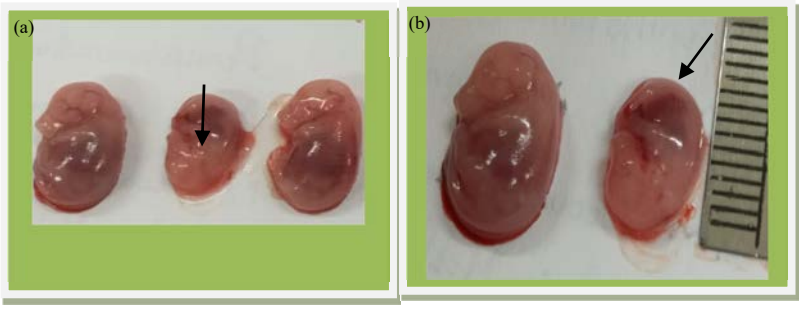

Fig. 2(a-b): Fetal malformation due to toxicity of patiwala leaf extract (*Lantana camara* Linn.) at one day of gestation, (a) Hemorrhagic fetus and (b) Dwarf fetus

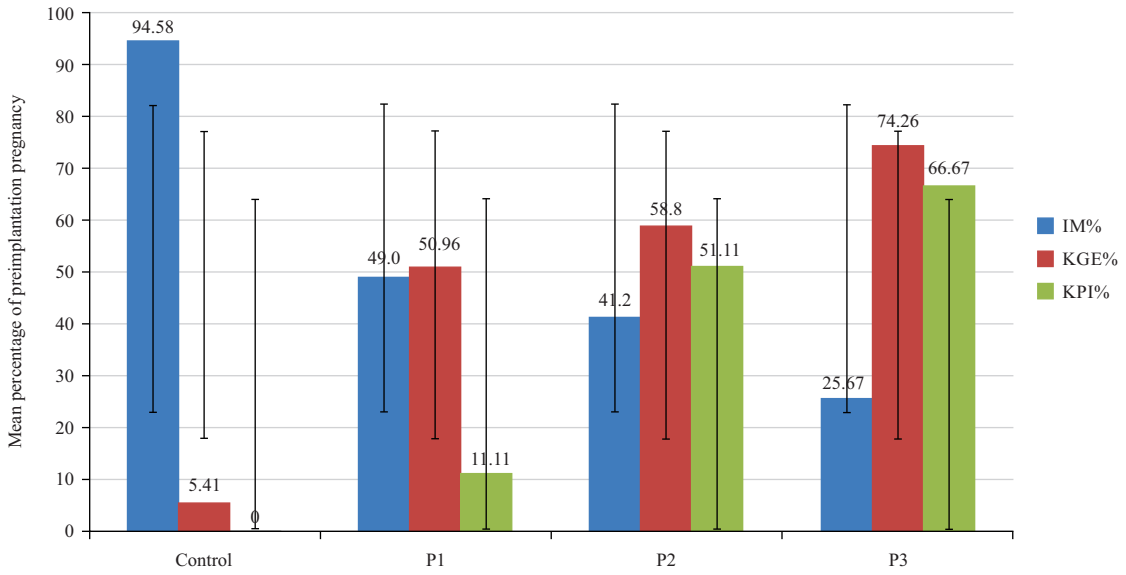

Fig. 3: Histogram of the percentage of pregnancy parameters in preimplantation rats (*Mus musculus* L.) after administration of patiwala leaf extract (*Lantana camara* Linn.)

of stunted fetuses and hemorrhagic fetuses. Dwarf fetuses have a small body size compared to normal fetuses. The malformation of the rat fetus due to the toxicity of patiwala leaf extract (*L. camara* Linn.) was shown in Fig. 2.

The existence of embryo malformations that were found, was caused by alkaloid compounds contained in *L. camara* leaves which caused cell death so that embryo growth was hampered. This is to what was stated by Bernstein *et al.*[37], that alkaloid compounds can interfere with cell replication, inhibit mitosis at the metaphase stage by inhibiting the formation of mitotic spindles resulting in chromosomes breaking, spreading or clumping and resulting in cell death, as a result, fetal development is hampered and finally become stunted. Disturbances in fluid viscosity and pressure, between blood plasma and the extra-capillary space, or between extra- and intra-embryonic fluids are all examples of foreign substances in the tissues. Due to this discrepancy, the fetus's blood arteries burst, resulting in bleeding[38].

A comparison of pregnancy parameters of rats (*Mus musculus* L.) at the preimplantation stage due to the toxicity of patiwala leaf extract (*L. camara* Linn.) for more details can be seen in Fig. 3.

Based on the comparison histogram of pregnancy parameters in preimplantation stage rats (*Mus musculus* L.), it shows that the toxicity of patiwala leaf extract (*L. camara* Linn.) on average causes pregnancy disturbances by reducing the percentage of implantation, increasing the percentage of gestational loss and increasing the percentage of post-implantation death. Antifertility compounds in patiwala leaf extract in the form of triterpenoid saponins cause disturbances in the hormones responsible for the pregnancy, such as estrogen and progesterone. This was to the

explanation[39], that triterpenoid and saponin compounds interfere with the function of the corpus luteum, resulting in a decrease in estrogen and progesterone.

The corpus luteum in the former follicle will produce estrogen and progesterone. Estrogen and progesterone act as negative feedback by decreasing FSH secretion so that no follicles grow in the ovary. The FSH (Follicle Stimulating Hormone) and LH (Luteinizing Hormone) are responsible for maintaining pregnancy for 1 to 12 days. The hormones FSH and LH that cannot be secreted result in the embryo not being able to survive due to obstruction of the supply of nutrients from the endometrial blood vessels, causing embryo death.

The toxicity of patiwala leaf extract (*Lantana camara* Linn.) in groups P1, P2 and P3 caused interference with the pregnancy of rats by inhibiting implantation and increasing the percentage of gestational loss (KGE) above 50%. The toxicity of patiwala leaf extract concentrations of 4 g/kg b.wt. (P2) and 6 g/kg b.wt. (P3) on the percentage of postimplantation mortality obtained above 50%. At a concentration of 2 g/kg b.wt. (P1), the percentage of postimplantation mortality was not significant but increased on average compared to the control group. This is probably caused by individual variations that are not homogeneous so the resulting data varies. Based on the results of this study it can be stated that patiwala leaf extract can be recommended as an anti-implantation raw material and can be developed to overcome the biological explosion of rat pest populations.

CONCLUSION

The conclusion of this study was that the toxicity of patiwala leaf extract (*Lantana camara* Linn.) is anti-implantation for pregnant rats because it causes implantation failure, increases gestational loss and postimplantation death. The results of this study are a source of new information regarding the use of the patiwala plant which is still considered a weed to be developed as an antifertility raw material by the community and to contribute to the success of government programs to maintain food security by controlling rat populations in a safe and environmentally friendly manner.

SIGNIFICANCE STATEMENT

Patiwala leaves (*Lantana camara* Linn.) have antifertility properties due to the presence of flavonoid, saponin and triterpenoid compounds which can inhibit FSH and LH secretion so that they are antiestrogenic which causes the uterine wall to decay and interferes with the implantation process in female rats. In Kendari City, Southeast Sulawesi Province, the patiwala plant is still considered a weed, its existence is quite numerous, it is easy to grow in marginal environmental conditions and its use as a source of antifertility material will not conflict with other interests. The results of this study are new findings in the form of scientific information that can be developed by farmers, the community and the government to control rat populations biologically.

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