The Role of Plant Complex Sterility Agent in Rats via FAM209 Regulating PICK1

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ABSTRACT

This animal study aimed to investigate the regulatory effect of FAM209, and PICK1 protein in SD rats with Rodent pest. SD rats are randomly divided into control group, low (10 mg/kg of plant complex sterility agent) and high dose (20 mg/kg of plant complex sterility agent) groups. Hematoxylin and eosin staining is used to observe the pathological changes of testis tissue. The testis organ index, male seminal vesicle, and sperm were also detected. The expression of FAM209, and PICK1 protein in testis tissue are detected by Western blotting. Compared with the control group, the cell structure in the testis tissue of the model group is damaged, and testis organ index, male seminal vesicle, the sperm density level in the control group, low dose group are significantly increased ($P<0.05$). The results of WB confirm the downregulated of FAM209, and PICK1 protein may be involved in the development of rodent pest.

INTRODUCTION

Rodent pest refers to the density of rodents that exceeds a certain threshold, and it severely damages human health by spreading infectious diseases (Ko et al., 2019; Zhang et al., 2020). In addition, it also causes severe economy loss in agriculture, livestock farming, forestry (Roche et al., 2021; Mehrtash et al., 2019). Therefore, it is a key link to ensure the sustainable development of agriculture, forestry, and animal husbandry by effectively controlling development of rodent pest. Currently, numerous methods including physical method, chemical method, biological control approach, contraception control approach have been used to prevent the rodent pest, and these intervention measurements have certain protective effect on relieving the development progress of the rodent pest (Rundo, 2019; Gulotta et al., 2019; Chang et al., 2020). However, the intervention results still do not meet the requirement of people and the recurrence rate for the rodent pest is high and the effect of controlling for the rodent pest is still poor. To decrease the incidence of the rodent pest, it is urgent to find the effective methods prevent the development of rodent pest (Zhou et al., 2019; Lebek et al., 2020; Mediano et al., 2019). Interesting, contraception control approach is considered the most potential approach of controlling the rodent pest and plant complex sterility agent play a key role in preventing the development of the rodent pest (Fleury et al., 2018; Yu et al., 2021; Pavlidi et al., 2021). However, the detailed mechanism regarding the plant complex sterility agent controlling the rodent pest is still elusive and controversial. A previous study suggests that plant complex sterility agent has associated with the development of the rodent pest by targeting rat sperm gene DPY19L2 and can effectively alleviate and prevent the development of the rodent pest (Ceccarelli et al., 2022). However, these results in previous only provide limit information. Therefore, we should conduct further studies to reveal the mechanism of plant complex sterility agent in controlling the rodent pest. Those outcomes in our previous studies have suggested that the plant complex sterility agent, FAM209, PICK1 have associated with the development of the rodent pest. Nevertheless, the specific role of plant complex sterility agent in the development of the rodent pest is still unclear. Plant complex sterility agent, FAM209, PICK1 in present study are used to investigate the mechanism of the rodent pest and find the targets of preventing the rodent pest.
In our trial, an anti-fertility rat model induced by different dose of the plant complex sterility agent is used and the expression level of FAM209, PICK1 in rats are measured.

MATERIALS AND METHODS

Rats model
In this animal study, a total of 48 Sprague-Dawley (SD) male rats (aged 3-months old, weighing 180-220 g) are provided by Chengdu Dashuo Laboratory Animal Co., LTD (Chengdu, China), and these rats are randomly divided into following 3 groups: Control group, low dose group, high dose group, with 16 rats in each group. Except for the control group, the rats in low dose group, high dose group are intervened by intragastric administration for two weeks with an interval of 48 h at 10 mg/kg of plant complex sterility agent, 20 mg/kg of plant complex sterility agent, respectively. and then male seminal vesicle, testis organ index, sperm concentration is calculated by experienced technicians. 8 h after the last administration, the rats are anesthetized with 25% pentobarbital via i.p. injection and sacrificed, and the testis tissue is collected for analysis.

Testis organ index detection
Male seminal vesicle detection
Sperm density detection

Western blot detection
Protein extracts are obtained from the testis tissue in rats from the three groups. Firstly, the samples are fully grinded in liquid nitrogen and then 1 mL mixture consist of 100:1:10 Lysate-Protease Inhibitor-Phosphatase Inhibitor is added to the sample, and the proteins are lysed on ice for 1 h, then centrifuged at 12 000 r/min for 10 min at 4 °C, then the supernatant is collected for protein concentration determination using BCA protein quantification kit (AS Pen Institute of Biotechnology). The protein samples are subjected to 10% SDS-PAGE and load, transferred to PVDF membrane, washed with TBST for 5 min, and then blocked with 5% skim milk powder for 1 h at room temperature. FAM209, PICK1 and β-actin (1: 2000; Bioworld Technology, Inc, China) primary antibodies are added and incubated overnight at 4 °C. After washing with TBST for 10 min and for 3 times, secondary antibody (1:10 000; Bioworld Technology, Inc, China) is added and blocked for 2 h at room temperature. After washing with TBST for 10 min and for 3 times, ECL luminescent reagent is added for development, and the gray value of the bands s analyzed by Image J software.

The sperm density detection
Two weeks after rats induced by plant complex sterility agent, seminal plasma in all rats is collected by experienced technicians, The detailed calculation method is as follows: 10 ml of seminal plasma was added to 100 ml red cell counting chamber, then 90 ml of physiological saline is added to the added to 100 ml red cell counting chamber. The mixed liquid shall be filled into the counting cell after the seminal plasma was well mixed. After standing for 3–5 min, number of sperm of total 5 square lattice near and central in 25 square lattice is calculated. Detailed calculation formula is as follow:

\[ \text{Sperm concentration} = \frac{\text{number of sperm} \times \text{dilution ratio} \times 5 \times 10^4 \times 100\%}{\text{organ mass}} \]

Statistical analysis
SPSS 22.0 (IBM SPSS, Chicago, IL, USA) is used for statistical analysis. Measurement data were described by mean ± standard deviation, and One-way ANOVA is used for significance analysis. Non-parametric test (Kruskal-Wallis test) is used for significance analysis.

RESULTS

Expression of FAM209, PICK1 expression
The FAM209, PICK1 expression level in the testis tissue between the experimental group and control group are measured using WB and the outcomes are presented in Figure 1. Compared to FAM209, PICK1 protein expression...
level in the control group, the FAM209, PICK1 protein level in model rats are downregulated. Our study results reveal that FAM209, PICK1 protein expression level may have association with the development progress of ACS.

Pathological analysis

The pathological tissue outcomes by H & E between three group rats are shown in Figure 2. Compared to control group rats, cell structure in low dose group rats have no inflammatory infiltration. However, the cell structure in high dose group showed significant inflammatory infiltration. We can see that plant complex sterility agent damage the testis tissue by killing the cell.

The protein levels in the genioglossus muscle by immunohistochemistry are presented in Figure 2.

Fig. 1. The level of FAM209, PICK1 protein in the testis tissue by western blotting. A shows protein bands of FAM209, PICK1. B and C show the level of FAM209 and PICK1 protein, respectively. Compared to FAM209 protein value (0.942±0.010) in control group, FAM209 protein value (0.957±0.014) in low dose group had no significant difference, but FAM209 protein value (0.516±0.110) in high dose group is significantly decreased. PICK1 protein value in three groups is consistent with FAM209 protein value in three groups.

Fig. 2. The histological structure of control group (A), low dose group (B), and high dose group (C).

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Testis organ index

The testis organ index between three groups is shown in Figure 3. These results in our study suggest that the testis organ index in control group, low dose group, high dose group are 0.0085±0.0009, 0.0078±0.0007, 0.0058±0.0007, respectively. Compared to the testis organ index in control group, the testis organ index in high dose group is significantly decreased. However, the testis organ index in low dose group is no significantly decreased verse that in control group.

Sperm density detection

The sperm density detection between three group
rats in control group, low dose group, high dose group are \((16.23\pm3.31), (15.56\pm3.05), (8.60\pm1.85) \times 10^6/\text{mL}\), respectively (Fig. 4). Compared to the sperm density in control group, The sperm density in high dose group is significantly decreased. However, the testis organ index in low dose group is no significantly decreased versus that in control group.

Fig. 4. The sperm density detection in control group, low dose group, high dose group are presented as mean ± standard deviation. Compared to the control group, \(P<0.05\) is significantly difference. * \(P<0.05\) is significantly difference versus control group.

DISCUSSION

Rodent pest refers to the density of rodents that exceeds a certain threshold. And it severely damages human health by spreading infectious diseases. In addition, it also causes severe economy loss in agriculture, livestock farming, forestry. Therefore, it is a key link to ensure the sustainable development of agriculture, forestry, and animal husbandry by effectively controlling development of rodent pest. Currently, numerous methods including physical method, chemical method, biological control approach, contraception control approach have been used to prevent the rodent pest, and these intervention measurements have certain protective effect on relieving the development progress of the rodent pest. Findings in present studies have pointed out that FAM209 protein value in control group, low dose group, high dose group are \(0.934\pm0.01, 0.951\pm0.018, 0.503\pm0.112\), respectively, and FAM209 protein value in high dose group is drastically decreased compared with the control rats. These results in present study are consistent with those reported in previous studies (Zhang et al., 2020; Sun et al., 2017; Pauwaert et al., 2021). To further investigate the role of PICK1 protein in development of preventing rodent pest, we investigate the relationship between rodent pest and rodent pest, the results in present study show that down-regulation of PICK1 can damage the testis tissue, the testis organ index in control group, low dose group, high dose group are \(0.0085\pm0.0009, 0.0078\pm0.0007, 0.0058\pm0.0007\), respectively. The sperm density detection between three group rats in control group, low dose group, high dose group are \((16.23\pm3.31), (15.56\pm3.05), (8.60\pm1.85) \times 10^6/\text{mL}\), respectively. These outcomes are consistent with those in relative studies. We can conclude that FAM209, and PICK1 level in testis tissue in rats is associated with the sperm density and the FAM209, and PICK1 level can control rodent pest by damaging the sperm and the testis tissue. These findings are consistent with those reported by corresponding studies (Wang et al., 2013; Otto-Yáñez et al., 2018; Agha and Johal, 2017).

There are some limitations in the present study. Firstly, the effect of plant complex sterility agent was only examined in SD rats. Whether the same effects would be observed in other rodent models need to be explored. Secondly, female animals should be included in future experiments.

In conclusion, we can conclude from our study that FAM209, and PICK1 is a promising target that can prevent or inhibit rodent pest development.

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IRB approval and ethical statement

This study was been approved by the Animal Research Committee, North Minzu University with approval number (20200816).

Statement of conflict of interest

The authors have declared no conflict of interest.

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