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Original Research Article

Toxicological testing of Fuganlin Oral Liquid on young Sprague-Dawley rats

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Abstract

Purpose: *To further evaluate the safety of Fuganlin Oral Liquid (FGLOL) for clinical application by conducting toxicological tests in young rats.*

Methods: *Based on the clinical dose for infants aged less than 1 year, 4-day-old Sprague-Dawley (SD) rats were orally administered FGLOL3.88, 11.64 and 38.75 g crude drug/kg for 18 days, followed by a 3-week withdrawal. This was followed by evaluating the effect of FGLOL on various growth and development indicators, nerve reflex function and spontaneous behavior of the rats. Based on the clinical dose for children aged 1 - 6 years, 15-day-old rats were orally administered FGLOL3.88, 11.64 and 38.75 g crude drug/kg for 31 days, followed by a 3-week withdrawal in order to evaluate the impact of the FGLOL on the development of reproductive organs and nervous systems in young rats. The effects of FGLOL on the safety of young rats were judged based on ophthalmic examination, physical examination, spontaneous behavior and other developmental effects.*

Results: *FGLOL did not cause animal death, and no significant toxicological changes were observed in the body weight, growth and development, and behavior of the young rats ($p < 0.05$). No observed adverse effect level (NOAEL) even after administering the higher dose at each stage. However, the results at each stage showed that oral administration of a large amount of FGLOL had a greater effect on normal food consumption of the rats ($p < 0.05$).*

Conclusion: *The findings indicate that Fuganlin Oral Liquid, a traditional Chinese medicine formulation, is safe in rats. However, clinical trials are required to acertain its safety in humans.*

Keywords: *Fuganlin Oral Liquid, Safety, Acute toxicity, Developmental toxicity, Juvenile rats*

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INTRODUCTION

Fuganlin Oral Liquid (FGLOL) is a Traditional Chinese Medicine Formula that has been approved for marketing and is mainly used to treat fever, cough, asthma, and sore throat caused by *qi* deficiency and cold in children [1]. In particular, there is a lack of toxicological studies on proprietary Chinese medicines used in children for the appropriate population. According

to the ICH S11 guidelines. For marketed TCM preparations that are intended to increase the scope of paediatric use or include paediatric use, companies should supplement their toxicological studies in young animals if there is a lack of systematic clinical and non-clinical safety evaluation information for paediatric use [2,3]. In previous studies, FGLOL was shown to exert good therapeutic effects on various types of asthma models in different young animal models, reducing airway hyper-responsiveness and controlling airway inflammation. It has also been shown to be effective in treating house dust mite-induced asthma in young rats, and ovalbumin-induced allergic asthma in young guinea pigs [4,5]. In this study, a juvenile animal study (JAS) was designed to evaluate the safety of FGLOL in young SD rats at different dosage cycles.

EXPERIMENTAL

Animals

Eighty-eight pregnant SD rats weighing 350 - 450 g were purchased from Hunan Slaike Jingda Laboratory Animal Co. Ltd, with Quality Certificate No. 1107271911001228. This trial was reviewed and approved for use by the Guangdong Lewwin Pharmaceutical Research Institute Animal Care and Use Committee (IACUC) (approval no. IA-SE2019032-01, 02, 03), and complied with the guidelines of AAALAC [6], and was conducted under NMPA GLP regulations. All environmental technical indicators met the environmental indicators of the institution.

Treatments

FGLOL was administered orally to 4-day-old rats at 3.88, 11.64 and 38.75 g of raw drug/kg for 18 d according to the clinical dose for infants up to 1 year of age, and was withdrawn for 3 weeks to further assess the effects of FGLOL on various growth and developmental indicators, neuroreflex function and spontaneous behaviour in rats. FGLOL was administered orally to 15-day-old rats at 3.88, 11.64 and 38.75 g of raw drug/kg for 31 d according to the clinical dose for infants up to 1-6 years old, and was withdrawn for 3 weeks to further assess the effects of FGLOL on various growth and developmental indicators, neuroreflex function and spontaneous behaviour in rats. FGLOL was administered orally to 40-day-old rats at 29.06, 58.13 and 116.25 g/kg of raw drug for 66 d according to the clinical dosage for 7-12 year-old infants. The effect of FGLOL on growth and development indicators, neuroreflex function and spontaneous behaviour was further evaluated by stopping the drug for 3 weeks.

Evaluation of treatment parameters

Clinical observations were made daily during the trial, focusing on lactation of the mother and suckling of the pups, turning, crawling, walking, gait, grooming behaviour and nesting behaviour. Young rats were weighed twice a week before weaning and suckling were recorded daily. Body weight and feed intake were measured once a week after weaning.

Physical growth and development, including body length, tail length, and limb length, were assessed weekly during the tests, while femoral bone density was determined at end-of-life test, using InAlyzer Dual-Energy X-ray Animal Body Composition Analysis System (Medikors Inc). Examinations of neurological reflex and growth development were performed as scheduled.

Statistical analysis

The study data were statistically analyzed using solutions Statistical Package for the Social Sciences (SPSS) 21 (IBM, Armonk, NY, USA). The data are presented as mean ± standard deviation (SD). Analysis of variance (F-test) between groups was performed before the experimental results were compared among groups. When the variances between groups were homogeneous, Student t-test (unpaired t-test) was used. When the variances of the groups were uneven, the corrected Student t-test was used for the statistical analysis.

RESULTS

Effect of FGLOL on clinical signs and behavior in young rats of different ages

Clinical observation of the animals showed that there were no significant abnormalities in suckling, feeding, excretion and coat colour in all groups of animals during the first and second phases of administration and recovery, and during the first and second treatment periods and recovery period. Moreover, significant abnormalities in feeding, excretion or coat color were observed during the third treatment period and recovery period. There were no statistically significant differences in turn-taking, crawling, walking, gait, grooming and nesting behaviour in the pups compared to the control group. Growth and development examination, as well as the neurological reflex development examination of the pups were normal (Table 1 and Table 2). The results of open-field activity test showed that at perioperative neurocognitive disorder (PND) 50, the development of nervous system of pups had reached mature stage. In the open-field test, the

range of movement of animals was mainly explored around the open field. The behavior of the animals in the negative group and in each treatment group was consistent, and no decreased activity or anxiety behavior was observed. At PND80, all animals underwent an eight-arm maze test to investigate learning and memory ability, and the results showed that the values of the indicators were close to each other in both the control and dosing groups, and no significant differences were observed (Figure 1 and Figure 2).

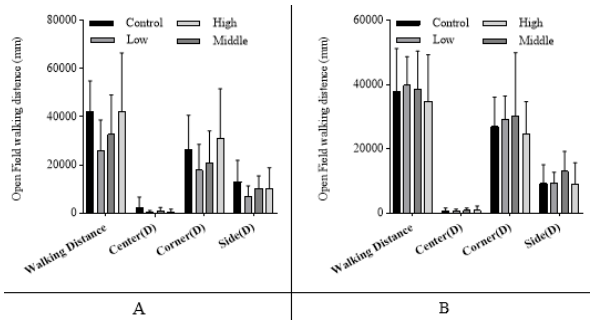

Figure 1: Open activity test exercise distance in the negative control and FGLOL-treated groups: (A) Male; (B) Female. ***Note:*** The values are expressed as mean ± SEM (n = 16 rats per gender). $P < 0.05$; $p < 0.01$ compared to control group

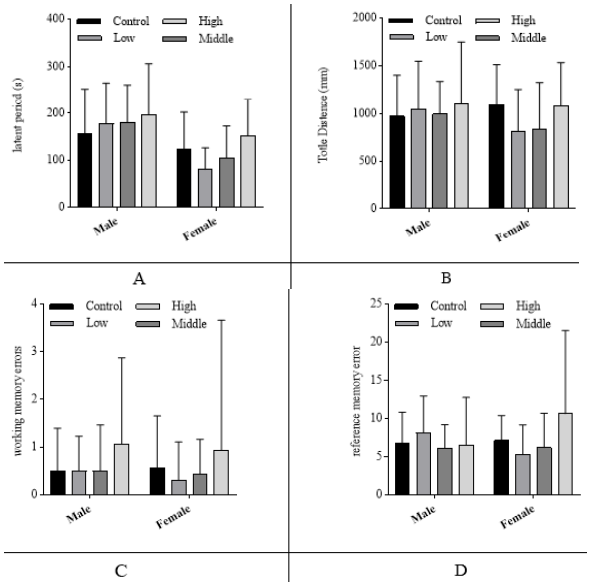

Figure 2: Eight-radial arm maze task in the negative control and FGLOL-treated groups: (A) Latent Period time; (B) total Distance. (C) Working memory errors; (D) Reference memory note errors: The values are expressed as mean ± SEM (n = 16 rats per gender). $P < 0.05$; $p < 0.01$ indicates statistical significance when compared with control group

Table 1: Compliance rate of growth and development indicators of offspring in Stage I and II

Test indicator	Stage 1 (% compliance)				Stage 2 (% compliance)			
	Control	***Low***	***Middle***	***High***	***Control***	***Low***	***Middle***	***High***
Lower incisor eruption (PND4)	100	100	100	100	100	100	100	100
Lana growth (PND13)	100	100	100	100	100	100	100	100
Opening eye (PND15)	100	100	100	100	100	100	100	100
Opening ear (PND20)	100	100	100	100	100	100	100	100
Orchiocatabasis (PND21)	100	100	100	100	100	100	100	100
Prepuce separation (PND42)	100	100	100	100	100	100	100	100
Vaginal opening (PND35)	100	100	100	100	100	100	100	100

Table 2: Achievement rate of neural reflex development in pups in Stages I and II

Test indicator	Stage 1 (% compliance)				Stage 2 (% compliance)			
	Control	***Low***	***Middle***	***High***	***Control***	***Low***	***Middle***	***High***
Surface righting reflex (PND5)	100	100	100	100	94	100	100	100
Cliff avoiding reflex (PND6)	100	100	100	100	100	100	100	94
Vibrissa localization reflex (PND8)	100	100	100	100	100	100	100	100
Olfaction Reflex (PND15)	100	100	100	100	100	100	100	100
Auditory shock reflex (PND16)	100	100	100	100	100	100	100	100
Air righting reflex (PND16)	100	100	100	100	100	100	100	100
Vision localization reflex (PND16)	100	100	100	100	100	100	100	100
Claw sting reflection (PND20)	100	100	100	100	100	100	100	100
Tail tenderness reflection (PND20)	100	100	100	100	100	100	100	100
Pupil focus reflex (PND21)	100	100	100	100	100	100	100	100

During the third phase of administration and the recovery period, no significant abnormalities were observed in the body weight of animals in all groups (Figure 3 and Figure 4). Feed consumption during the first phase was measured during the withdrawal period, and it was observed that the feed consumption of animals in each FGLOL dose group was increased compared with the control group.

During the second and third treatment periods, the feed intake of animals in each FGLOL treatment group decreased compared with the control group, and after the discontinuation of the drug, the amount of food consumed increased significantly compared to the control group, which shows that the oral administration of large amounts of FGLOL has a greater impact on the normal feeding of rats, and there is a certain dose-related relationship with the consistency of the drug, and after discontinuation of the drug, the animals will consume more feed in order to maintain the feeling of a full stomach, but the magnitude of the above changes did not exceed 20 %, and there is no corresponding effect on body weight, and this phenomenon has less significance for clinical guidance (Figure 5 and Figure 6).

In the group of FGLOL 11.64, 38.75g/kg: In the first stage, body length and limb length were significantly decreased when compared with the control group during the pre-dose period, which correlated with body weight changes and returned to normal after discontinuation. During the second and third withdrawal periods, significant decreases in body length and limb length were observed at some time points in each dose group when compared with the control group, but no weight-related changes were observed (Figure 7, Figure 8 and Figure 9). At different ages, all animals underwent bone mineral density testing (at an early age) or femur (at an adolescent age), which showed that in PND $_{21}$ ~ PND $_{46}$, the bones of young rats were in a rapidly developing stage, and bone mineral density gradually increased. After reaching PND$_{66}$, bone development was basically completed. The bone mineral density of rats remained at a relatively stable level until adulthood. There was no significant difference in bone mineral density values among the groups, and the drug had no significant effect on bone development (Figure 10).

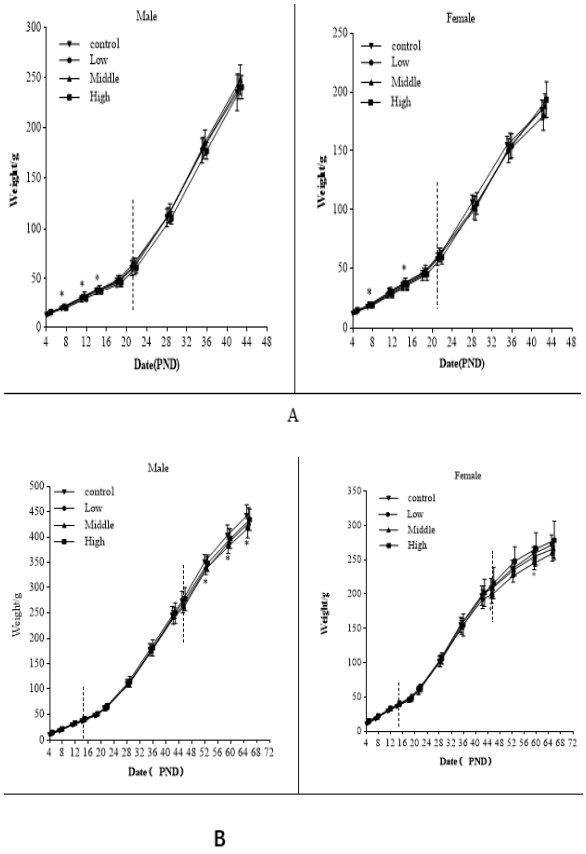

Figure 3: Body weight changes in the negative control and FGLOL-treated groups during the treatment period: (A) PND $_{4}$ -PND $_{21}$ during the treatment period in stage 1, PND $_{22}$ -PND $_{42}$ during the recovery period; (B) PND $_{15}$ -PND $_{45}$ during the treatment period in stage 2, PND $_{46}$ -PND $_{61}$ during the recovery period

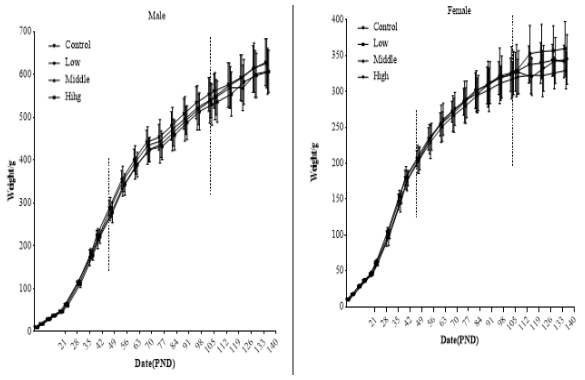

Figure 4: Dosing Phase PND $_{40}$ -PND $_{105}$, Recovery Phase PND $_{106}$ -PND $_{136}$. Note: The values are expressed as mean ± SEM (n = 16 rats per gender for dosing period, n = 8 rats per gender for withdrawal period at each stage). $P < 0.05$; $p < 0.01$ compared to control group

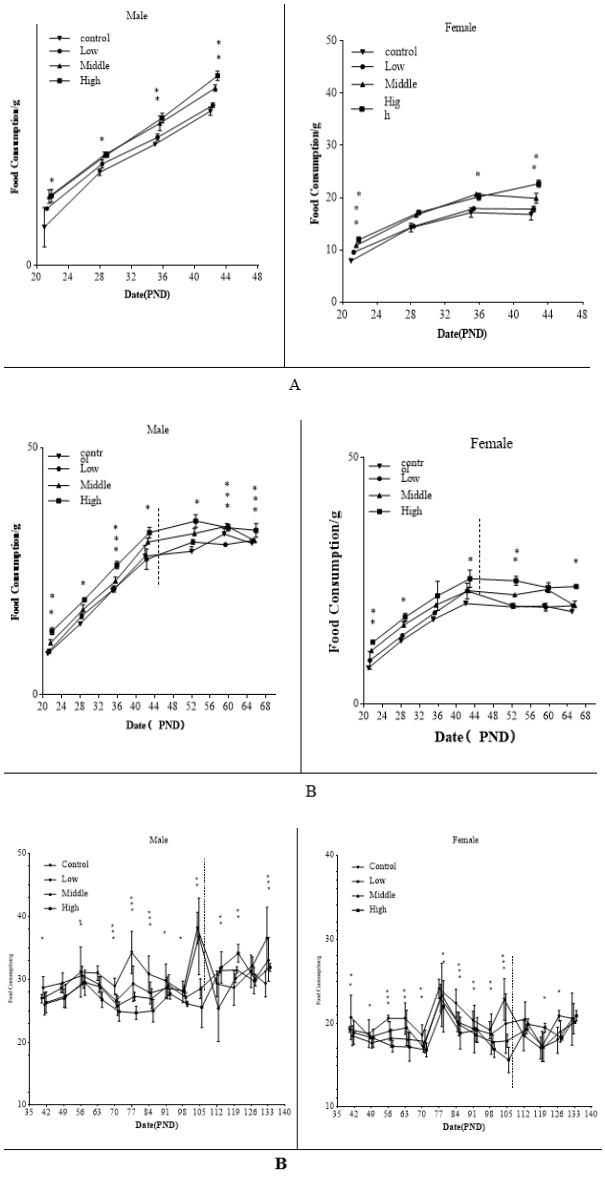

Figure 5: Changes in Feed consumption in the negative control group and FGLOL-treated group during the period (A) stage 1 recovery PND $_{22}$ -PND $_{42}$; (B) stage 2 treatment PND $_{21}$ -PND $_{45}$, recovery PND $_{46}$ -PND $_{61}$

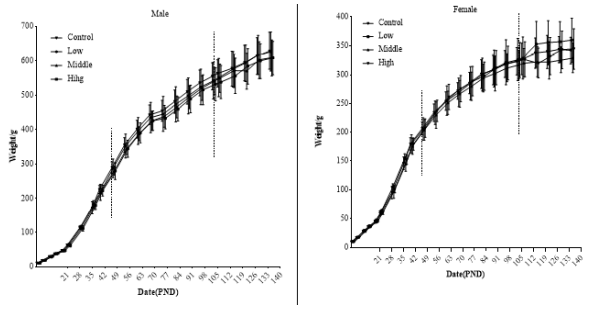

Figure 6: Dosing PND $_{40}$ -PND $_{105}$, Recovery PND $_{106}$ -PND $_{136}$. The values were expressed as mean ± SEM (n = 16 rats per gender for dosing period, n = 8 rats per gender for withdrawal period in each stage). $P < 0.05$; $p < 0.01$ significant compared to control group

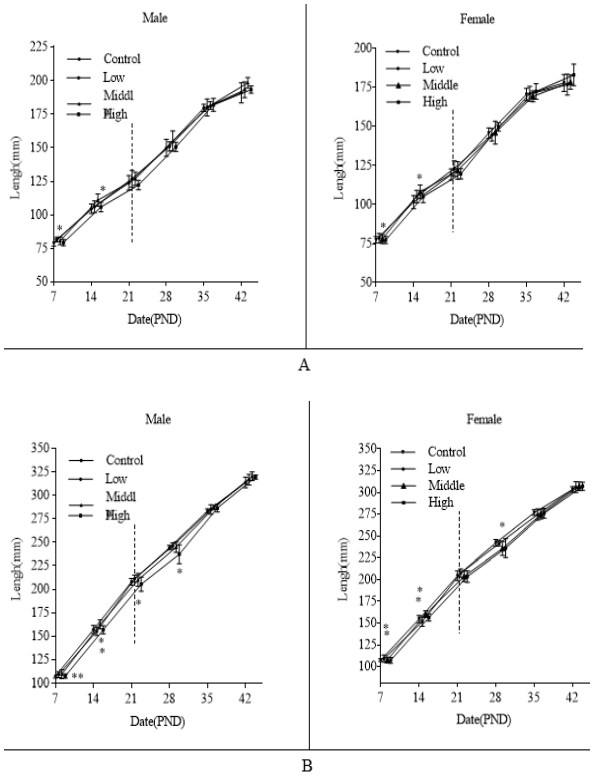

Figure 7: The body length and limbs of pups at various stages were longer in the negative control group and FGLOL-treated groups: stage 1 (A)Variation in body length between the two groups, (B)Variation in limbs length between the two groups; stage 2

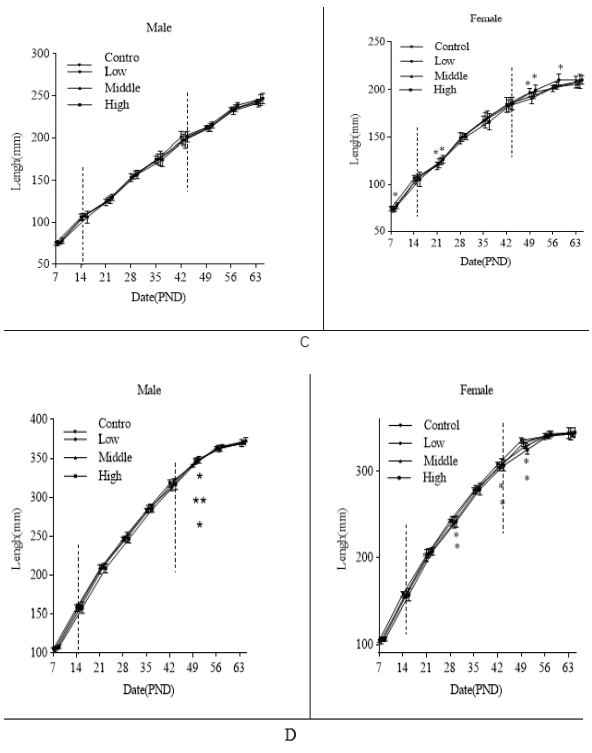

Figure 8: (C) Variation in body length between the two groups, (D) Variation in limbs length between the two groups; stage 3

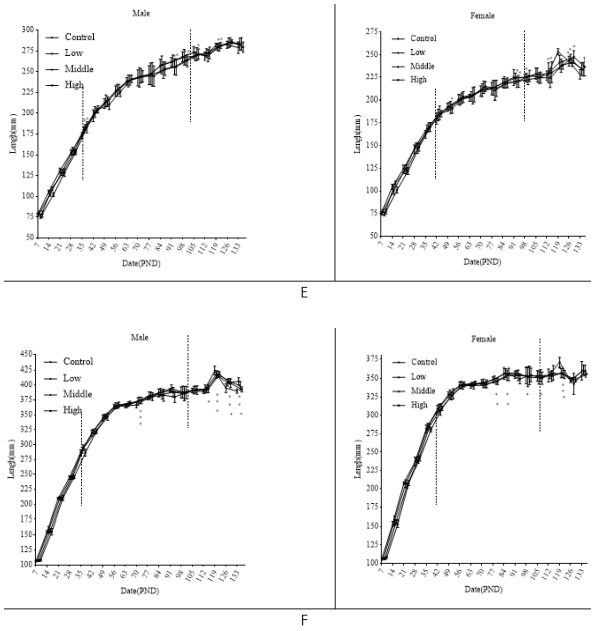

Figure 9: (E) Variation in body length between the two groups, (F) Variation in limbs length between the two groups. The values are expressed as mean ± SEM (n = 16 rats per gender for dosing period, n = 8 rats per gender for statistically withdrawal period in each stage). $p < 0.05$; $p < 0.01$ significant compared to control group

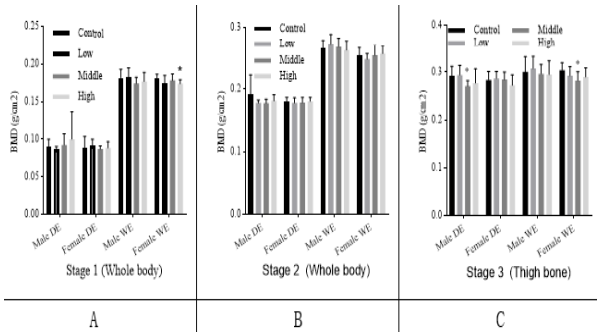

Figure 10: Bone mineral density of pups at various stages in the negative control and FGLOL-treated groups: (A) stage 1 D Withdrawing End (DE) on PND $_{22}$, Withdrawal End (WE) on PND $_{43}$; (B) stage 2 DE on PND $_{46}$, WE on PND $_{67}$; (C) stage 3DE on PND $_{106}$, WE on PND $_{136}$. ***Note:*** The values are expressed as mean ± SEM (n = 16 rats per gender for dosing period, n = 8 rats per gender for statistically withdrawal period in each stage). $p < 0.05$; $p < 0.01$ significant compared to control group

DISCUSSION

Fuganlin Oral Liquid (FGLOL) was marketed in China in the 1990s. In more than 20 years of clinical use since its market launch, children are usually cured after 2-3 doses [7], and if used for another 2-3 weeks, they can stop catching colds for a longer period of time. 70 % of cough symptoms mostly subside within 24 - 48 h after medication. In recent years, FGLOL has been found to be effective in the treatment of cough-variant asthma, and is intended to be used for symptomatic treatment of asthma indications in children [8,9]. The clinical medication cycle is relatively short, but it covers a wide range in children, from 1 year old to 12 years old. In the JAS trial, if administered on an all-age continuous basis, dosing periods beyond the therapeutic cycle may produce less toxic reactions and may pose a barrier to clinical use of the drug [10,11]. Therefore, the little piglets delivered by the same batch of dams were used in this study, in order to maintain the consistency of animal background, and short-term, mid-term and medium-term drugs were administered at different initial age groups based on different intended clinical medication cycles and ages of children [12]. Based on the principle of cross-coverage of developmental age, a three-stage JAS trial was designed to objectively evaluate the toxicological test of F GLOL in young animals.

In the three-stage study, it was found that using litters from the same dams with the same date of conception, allowed for better control of the growth background of the litters, more consistent results in all growth and development indicators, and easier detection of the toxic effects of the drug. Moreover, animals with the same pregnancy date were selected. The delivery date controlled at 3 days before and after, which meet the requirements of using pups of the same age for 3 - 4 trials.

The overall study could reduce the number of dams used, which was more in line with the requirements of animal welfare reduction and optimal design. In the Phase III study, the age of initiation of dosing was the same as the age of the conventional repeated dose toxicity test. This study therefore combines the two studies, simultaneously monitoring JAS and repeat toxicity indicators, and the dose administered was increased compared to Phase I and Phase II. The study results also showed that F GLOL had good safety and no drug-related significant toxicity occurred. In addition, the study results also suggested that it was inappropriate to administer too dense drugs to young animals. The results from all phases of the study show that the oral administration of large amounts of FGLOL has a greater effect on normal feeding in rats, with a dose-dependent relationship with the consistency of the drug, and that the animals will consume more feed to maintain a full stomach after discontinuation of the drug. This effect was more pronounced around the time of weaning and was reflected in the effect on body weight gain and thus on physical development, which

was less pronounced in the third phase of post-weaning administration, considering that this is due to the specificity of the animal test and has less significance for clinical guidance.

In summary, the oral administration of FGLOL started at perioperative neurocognitive disorder (PND), 4 (approximately equivalent to human infancy, < 1 year of age) for 18 consecutive days at a nontoxic effect level (NOAEL) of 38.75 g crude drug/kg in young SD rats, equivalent to 19.70 times the daily pediatric dose in clinical practice within 1 year of age. FGLOL was administered orally from PND15 (approximately equivalent to human infancy, i.e., 1 to 2 years of age) for 31 consecutive days at a NOAEL of 38.75 g raw drug/kg, which is equivalent to 16.77 times the clinical daily dosage for children up to 6 years of age. FGLOL was administered orally starting at PND $_{40}$ (approximately equivalent to human adolescence) for 66 consecutive days, giving a NOAEL of 116.25 g crude drug/kg, which is clinically equivalent to 25.7 times the daily dose in children aged 7 to 12 years.

CONCLUSION

The safety of Fuganlin Oral Liquid, a traditional Chinese medicine in rats has been demonstrated in this study. Moreover, relevant data to guide the safe application of FGLOL in clinical practice has been presented. However, clinical trials are required in this regard prior to use in clinical practice.

DECLARATIONS

Acknowledgements

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Ethical approval

None provided.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Jianmin Guo and Yuankeng Huang contributed equally to this work.

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