SUBCHRONIC ORAL TOXICITY ASSESSMENTS OF "CAN HUYET VUONG" IN THE EXPERIMENT

Pham Thi Van Anh[1], Bui Thi Huong Thao[1], Dinh Thi Thu Hang[1]

[1] Department of Pharmacology, Hanoi Medical University
Corresponding author: **Dinh Thi Thu Hang**
Email: dinhthuhang@hmu.edu.vn
Received date: 09/09/2024
Reviewed date: 30/09/2024
Accepted date: 30/09/2024

SUMMARY

Nowadays, there has been a current trend for researchers to find out new natural ingredients which were safe and still effective in the treatment of diseases. "Can Huyet Vuong" was a herbal-derived product with two main ingredients including Curcuma longa L. (fresh turmeric) and Apis mellifera L. (honey bee) used as an oral medication. So far, the safety of this product has not been reported yet in Vietnam as well as in the world. Thus, this study was designed to assess the subchronic toxicity of "Can Huyet Vuong" in Wistar rats. The method used in this study followed the guidance of World Health Organization in rats with 2 oral doses of 1.8 g/kg b.w/day and 5.4 g/kg b.w/day for 30 days. As a result, "Can Huyet Vuong" did not caused deleterious impacts on general condition, hematological indexes, hepato-renal functions and microscopic images of liver and kidney. Hence, "Can Huyet Vuong" does not appear to produce subchronic toxicities in experimental animals.

Key words: *"Can Huyet Vuong", subchronic toxicity, Wistar rats.*

1. INTRODUCTION

The natural world has been a source of medicinal agents from ancient times and plenty of traditional medicines used in many countries worldwide have been formed from medicinal plants. [1] The exclusive use of herbal drugs for the prevention and treatment of a variety of diseases continues due to easy access, fewer adverse events, and economic reasons. According to the World Health Organization (WHO), traditional medicines are used by up to 80% of developing country populations for their primary health care. However, the biggest concern of medicinal plant use was the lack of evidence-based approaches and the lack of toxicological profiling of herbal preparations. Thus, evaluating their toxicity plays an important role in recognizing the toxicity of drug-derived herbal medicine, in helping to characterize them, to evaluate their risk for humans, and in recommending whether further human studies should be conducted and suggest appropriate dose levels for clinical trials. [2]

Toxicity refers to unwanted effects on the body's biological systems. In order to assess potential toxicity, it is crucial to choose the correct system, since no effects can otherwise be seen. Toxicity of a substance can be influenced by many factors, such as the route of exposure (topical application, oral administration, inhalation, or injection); the time of exposure (acute, subchronic, or chronic use); the number of exposures (a single dose or multiple doses over a period of time); the physical form of the toxin (solid, liquid, or gas); the organ system involved (cardiovascular, urinary, respiratory, nervous, hematopoietic system); and even the genome and physiological structure of the target cells or organisms. [3].

"Can Huyet Vuong" was a herbal-derived product containing two main

ingredients *Curcuma longa L.* (fresh turmeric) and *Apis mellifera L.* (honey). *Curcuma longa* L., was well known as turmeric, belongs to the Zingiberaceae family and used for traditional medicine as a remedy for various diseases. *Curcuma longa* L. has been used for supporting to treat liver obstruction and jaundice with oral administration, or ulcers and inflammation with topical administration. Moreover, it is used in other diseases such as gastrointestinal disorder, bronchitis, tumor, and hepatic disorders. [4] Curcuminoids from *Curcuma longa* L. have been assessed in a large number of *in vitro* and *in vivo* studies to exhibit potential biological activities. [5] Various beneficial effects of curcuminoids has been illustrated, including antioxidant and anti-cancer properties, antimicrobial properties, cardioprotective, hepatoprotective and neuroprotective properties. Honey is a product derived natural resources created by honey bees and stingless bees. Honey from both types of bee contain different types of phenolic and flavonoid compounds with variable biological activities and clinical applications. So far, honey can be used effectively for wound healing. Both honey bee and stingless bee honey were used for traditional and modern mecicine with various properties– such as the treatment of liver disorders, gastrointestinal tract diseases, neurological disorders and fertility disorders. [6] However, so far, there have been no reports available on the toxicity of a combination product from these components. Therefore, the present study aimed to validate the subchronic toxicity of “Can Huyet Vuong” in experimental animals.

2. MATERIALS AND METHODS

2.1. The preparation of “Can Huyet Vuong”

“Can Huyet Vuong” was manufactured by Vi Dieu Nam Medicine And Pharmacy Limited Company. This product contained two main ingredients as *Curcuma longa L.* (fresh turmeric) and *Apis mellifera L.* (honey bee). This product was prepared and offered in form of liquid extract.

2.2. Chemicals and laboratory equipments

Kits for testing enzymes and metabolites in blood: ALT (alanin aminotransferase), AST (aspartat aminotransferase), total bilirubin, albumin, total cholesterol, creatinine kits from Hospitex Diagnostics (Italy) và DIALAB GmbH (Austria) were used for Screen Master machine of Hospitex Diagnostics (Italy). Blood-testing solutions ABX Minidil LMG of ABX Diagnostics were used for Vet abcTM Animal Blood Counter. Chemicals for tests and histopathological examination.

2.3. Experimental animals

Healthy *Wistar* rats (180 ± 20 g) were used in this study. The animals were housed in cages (groups of ten rats/cage) under the standard conditions (temperature 25^0C ± 2^0C and relative humidity 80% ± 10%), 12 hours dark/light time. We fed the mice with standard animal feed and allowed free access to water. They were acclimated to housing for at 10 days prior to investigation at the Department of Pharmacology, Hanoi Medical University.

2.4. Subchronic toxicity study

Subchronic toxicity study were carried out according to WHO guidelines. [7]

The study was carried out in a continuous 30-day period. *Wistar* rats were divided into three groups of ten animals:

- Group 1 (control) was administered 1 mL distilled water/100 g b.w/day;

- Group 2 was administered “Can Huyet Vuong” at the dose of 1.8 g/kg b.w/day (equivalent to human recommended dose, conversion ratio 6);

- Group 3 was applied “Can Huyet

Vuong" at the dose of 1.8 g b.w/kg/day (3 times as high as the dose at group 2).

Animals were treated daily by oral route of administration once a day in the morning for 30 days and were observed once daily to detect signs of toxicity.

The signs and indexes were checked during the study including:

- General condition consists of mortality and clinical signs.
- Body weight changes
- Hematopoietic function: red blood cells (RBC), hemoglobin (HGB), hematocrit, total white blood cells (WBC), WBC differentials, platelet count (PLT).
- Serum biochemistry: aspartate amino transferase (AST), alanine amino transferase (ALT), total bilirubin, albumin, total cholesterol and creatinine levels.

The parameters were checked at these following times: before treatment, 15 days after treatment, and 30 days after treatment. At the end of experiment, all animals were subjected to a full gross necropsy. Liver and kidney of 30% rats of each group will be removed for histopathology examinations. The micro-histological examination was carried out at the Department of Pathology, Duc Giang General Hospital.

2.5. Statistical analysis

Data were analyzed using Microsoft Excel software version 2016. The levels of significance between the experimental groups and the control group were made using student's t-test and Avant-après test. Data was shown as mean ± standard deviation. All data were considered significantly at $p < 0.05$.

3. RESULTS

3.1. General condition

Animals had normal locomotor activities and good feedings. None of the animals in all treated groups showed any macroscopic or gross pathological changes when compared with the control group.

3.2. Body weight changes

Table 1. The effect of "Can Huyet Vuong" on body weight changes

Time	Body weight (g)		
	Group 1	**Group 2**	**Group 3**
Before treatment	162.00 ± 13.17	161.00 ± 14.49	160.00 ± 25.82
15 days after treatment	181.00 ± 17.92$^{\Delta}$	179.00 ± 22.34$^{\Delta}$	184.00 ± 34.06$^{\Delta}$
30 days after treatment	194.00 ± 11.74$^{\Delta}$	186.00 ± 24.59$^{\Delta}$	193.00 ± 40.84$^{\Delta}$

* $p < 0.05$, ** *p < 0.01,* *** *p < 0.001 compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *p < 0.01,* $^{\Delta\Delta\Delta}$ *p < 0.001 compared with the time point "before treatment"*

Table 1 showed that after 15 days and 30 days of treatment, there was a significant increase in body weight at all groups as compared with the time point "before treatment" ($p < 0.05$). No significant differences were observed between groups treated "Can Huyet Vuong" and control group ($p > 0.05$).

3.3. Effect on hematological examination

There was no significant difference in hematocrit, hemoglobin level, MCV and platelet count between groups treated "Can Huyet Vuong" and group 1 ($p > 0.05$) in Table 2.

In terms of red blood cell count, there was a significant decrease in the group treated "Can Huyet Vuong" at the dose of 1.8 g/kg/day as compared with the control group and the time point "before treatment" but red blood cell count was stil in normal range.

Table 2. Effect of "Can Huyet Vuong" on hematopoietic function

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
Red blood cell count (T/L)	Group 1	11.57 ± 0.97	12.29 ± 0.50	12.18 ± 0.82
	Group 2	12.28 ± 1.20	11.24 ± 0.47***$^{\Delta}$	10.98 ± 0.62**$^{\Delta\Delta}$
	Group 3	11.38 ± 1.13	11.56 ± 1.16	11.36 ± 0.98
Hemoglobin level (g/dL)	Group 1	13.69 ± 0.77	13.12 ± 1.05	13.79 ± 1.10
	Group 2	13.71 ± 1.45	12.42 ± 1.20	12.38 ± 2.51
	Group 3	13.64 ± 0.70	12.49 ± 1.40	12.88 ± 1.41
Hematocrit (%)	Group 1	61.59 ± 4.91	62.66 ± 4.16	60.67 ± 5.17
	Group 2	60.77 ± 4.94	58.59 ± 5.10	57.73 ± 4.58
	Group 3	61.65 ± 3.99	58.71 ± 4.30	58.50 ± 5.60
MCV (fL)	Group 1	53.20 ± 2.30	51.20 ± 2.74	51.10 ± 3.07
	Group 2	53.50 ± 3.72	51.20 ± 2.66	51.00 ± 3.13
	Group 3	54.00 ± 2.62	51.70 ± 3.02	52.90 ± 1.29
Platelet count (G/L)	Group 1	656.20 ± 175.28	707.80 ± 148.46	756.10 ± 143.25
	Group 2	635.40 ± 90.97	653.40 ± 164.26	702.10 ± 62.67
	Group 3	638.40 ± 80.53	669.60 ± 175.12	675.00 ± 134.92

MCV: Mean corpuscular volume

* p < 0.05, ** *p < 0.01, *** p < 0.001 compared with control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ *p < 0.01, $^{\Delta\Delta\Delta}$ p < 0.001 compared with the time point "before treatment*"

Table 3. Effects of "Can Huyet Vuong" on total WBC count and WBC differentials

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
Total WBC count (G/L)	Group 1	9.52 ± 1.31	8.98 ± 1.23	10.83 ± 1.74
	Group 2	9.01 ± 2.05	9.54 ± 2.79	9.91 ± 2.21
	Group 3	9.58 ± 2.17	8.22 ± 1.80	11.31 ± 1.43
Lymphocytes (%)	Group 1	74.27 ± 6.33	69.26 ± 7.40	71.68 ± 5.88
	Group 2	75.47 ± 4.23	73.07 ± 7.39	73.44 ± 5.11
	Group 3	74.46 ± 5.78	68.83 ± 5.38	71.09 ± 5.57
Neutrophils (%)	Group 1	12.07 ± 3.48	16.50 ± 4.50	13.18 ± 4.21
	Group 2	11.86 ± 2.50	14.17 ± 2.99	15.20 ± 3.67
	Group 3	12.98 ± 2.77	16.85 ± 4.23	15.71 ± 4.01

WBC: white blood cells

* p < 0.05, ** *p < 0.01, *** p < 0.001 compared with control group (group 1)*

$^{\Delta}$ p < 0.05, $^{\Delta\Delta}$ *p < 0.01, $^{\Delta\Delta\Delta}$ p < 0.001 compared with the time point "before treatment*"

Table 3 demonstrated that at all time points, there was no significant difference in total WBC count, lymphocytes and neutrophils at groups treated “Can Huyet Vuong” as compared with group 1 and the time point “before treatment” ($p > 0.05$).

3.4. Effect on liver parameters

There were no significant diferences in total bilirubin, albumin concentration and total cholesterol concentration between groups treated “Can Huyet Vuong” and group 1 ($p > 0.05$). However, after 30 days of treatment, at group 2 and 3, aspartate amino transferase (AST) and alanine aminotransferase (ALT) levels increased significantly as compared with group 1 and the time point “before treatment” ($p < 0.001$).

Table 4. Effects of “Can Huyet Vuong” on liver parameters

Parameters	Group	Before treatment	15 days after treatment	30 days after treatment
AST level (UI/L)	Group 1	79.40 ± 14.06	70.90 ± 8.71	70.90 ± 15.47
	Group 2	91.00 ± 20.35	82.40 ± 18.42	72.90 ± 12.82
	Group 3	92.40 ± 19.55	79.90 ± 12.43	83.90 ± 21.34
ALT level (UI/L)	Group 1	31.80 ± 6.14	29.40 ± 7.24	31.00 ± 7.77
	Group 2	38.50 ± 9.05	36.20 ± 8.93	34.90 ± 7.39
	Group 3	35.30 ± 8.51	29.20 ± 8.93	38.00 ± 8.15
Total bilirubin (µmol/L)	Group 1	9.11 ± 0.67	9.50 ± 0.63	9.01 ± 0.34
	Group 2	8.93 ± 0.56	9.30 ± 0.71	8.94 ± 0.41
	Group 3	8.88 ± 0.43	9.47 ± 0.88	8.99 ± 0.48
Albumin concentration (g/dL)	Group 1	3.05 ± 0.16	3.42 ± 0.55	3.36 ± 0.37
	Group 2	3.21 ± 0.35	3.49 ± 0.39	3.04 ± 0.34
	Group 3	3.09 ± 0.39	3.21 ± 0.31	3.01 ± 0.38
Total cholesterol concentration (mg/dL)	Group 1	1.66 ± 0.25	1.75 ± 0.29	1.50 ± 0.17
	Group 2	1.65 ± 0.30	1.52 ± 0.24	1.42 ± 0.25
	Group 3	1.69 ± 0.21	1.68 ± 0.38	1.47 ± 0.26

* $p < 0.05$, ** *$p < 0.01$, *** $p < 0.001$ compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *$p < 0.01$,* $^{\Delta\Delta\Delta}$ *$p < 0.001$ compared with the time point “before treatment”*

3.5. Effect on kidney function

Table 5 illustrated that “Can Huyet Vuong” caused no significant differences in serum creatinine level between groups treated “Can Huyet Vuong” and group 1 ($p > 0.05$).

Table 5. Effects of “Can Huyet Vuong” on serum creatinine level

Days	Creatinine level (mg/dl)		
	Group 1	***Group 2***	***Group 3***
Before treatment	75.00 ± 6.90	74.22 ± 6.88	77.22 ± 11.28
15 days after treatment	74.60 ± 6.31	75.00 ± 5.96	79.70 ± 6.88
30 days after treatment	75.50 ± 6.72	74.40 ± 6.15	76.40 ± 4.25

* $p < 0.05$, ** *$p < 0.01$, *** $p < 0.001$ compared with control group (group 1)*

$^{\Delta}$ $p < 0.05$, $^{\Delta\Delta}$ *$p < 0.01$,* $^{\Delta\Delta\Delta}$ *$p < 0.001$ compared with the time point “before treatment”*

3.6. Histopathological examination

No gross lesions or changes in size was observed when subjected all experimental rats to a full gross necropsy which examined of the hearts, livers, lungs, kidneys and abdominal cavities. There was no significant difference in histopathological examination of liver and kidney between groups treated “Can Huyet Vuong” and control group after 30 days of treatment (Figure 1 and 2).

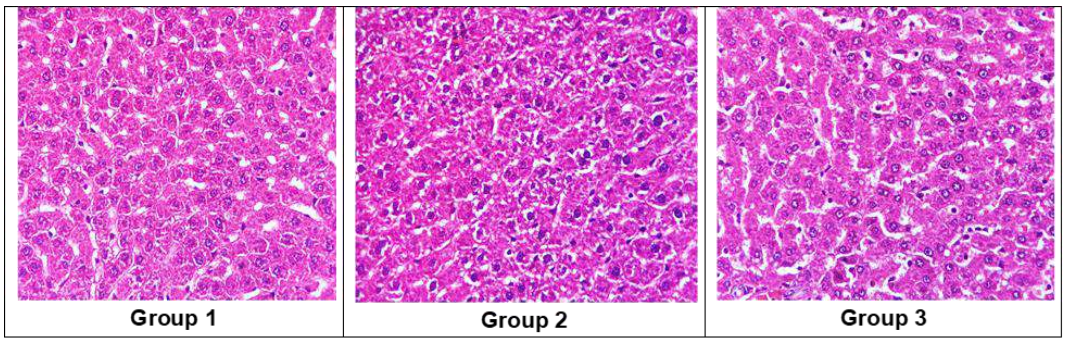

Figure 1. Histopathological images of liver (HE × 400)

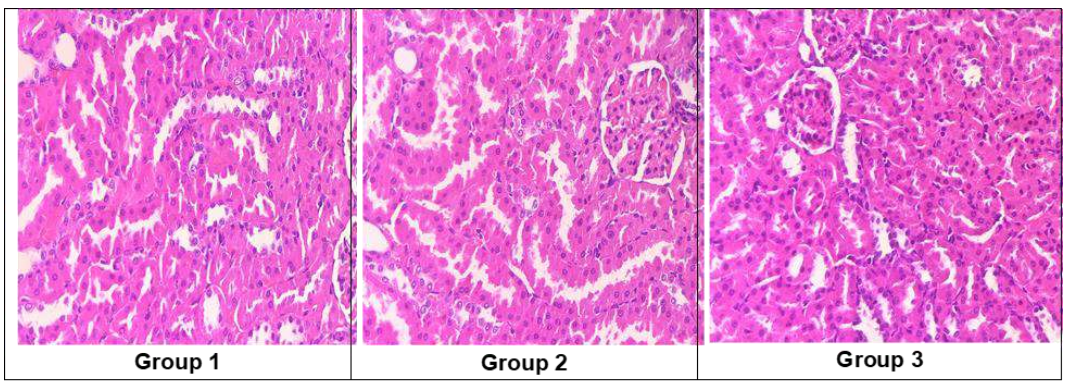

Figure 2. Histopathological images of kidney (HE × 400)

4. DISCUSSION

Toxicity is the negative impact to which a substance can harm the human body system. Toxicity can refer to the influence on a cell (cytotoxicity), an organ (e.g. nephrotoxicity or hepatotoxicity), or the whole organism. To assess the safety of drugs and herbal products for clinical use, various experimental animal models should be selected to conduct the toxicological evaluation for predicting toxicity and to provide recommendations for selecting ‘safe’ therapeutic dose levels in clinical. A subchronic toxicity study provides information on the impacts of repeated administration and can assess the need for further longer-term studies. [7], [8] Subchronic studies evaluate the undesirable effects of continuous exposure of traditional medicines or drugs over a part of the average life span of experimental animals, such as rodents. In particular, data from these studies provide toxicity information on target organs. [9]

The body weight changes serve as a sensitive index of the general health status of the animal. [10] Weights were observed in all animals treated with “Can Huyet Vuong”. It can be stated that “Can Huyet Vuong” did not interfere with the normal metabolism of animals as corroborated by the nonsignificant change from the control group.

The hematopoietic system is highly sensitive to influences from toxic compounds and is a vital index of physiological and pathological status in the body. Furthermore, when the data are translated from animal studies, the information about changes of the hematology system has higher predictive value for human toxicity. [7] After 15 days and 30 days of the treatment, there were no significant differences in hematocrit, hemoglobin level, platelet count, total WBC count and WBC differentials between groups treated “Can Huyet Vuong” with the control group. In terms of red blood cell count, there was a significant decrease in the group treated “Can Huyet Vuong” at the dose of 1.8 g/kg/day as compared with the control group and the time point “before treatment”, however, red blood cell count was stil in normal range. So it can be concluded that the administration of “Can Huyet Vuong” did not affect the hematological profile and blood formation process.

Evaluation of kidney and liver functions plays an important role in the toxicity evaluation of plant extracts as they are both necessary for the survival of a living organism. Analyses of biochemistry indexes were performed to assess the possible alterations in the function of the hepato-renal system impacted by herbal medicines. [7] The liver releases aspartate transaminase (AST) and alanine transaminase (ALT) and an elevation in serum levels of these enzymes is an indicator of hepatocellular damage. [11] The results showed that there was no subtantial change in the AST and ALT levels between the group treated “Can Huyet Vuong” and the control group. These results indicated that “Can Huyet Vuong” had no deleterious effect on liver function.

Creatinine level is a critical important index used in assessing the function of the kidney.**Error! Reference source not found.** No significant differences were observed in the creatinine level between control group and groups treated “Can Huyet Vuong” at various dose levels ($p > 0.05$). Therefore, “Can Huyet Vuong” did not affect kidney function.

The histopathological examination revealed the alteration in the microstructure of organs and the signs of the disease when viewed under brightfield microscopy. The further histological study could provide additional information regarding the hepatotoxicity and nephrotoxicity of “Can Huyet Vuong”. Our study showed that there was no significant difference in histopathological examination of the liver and kidney between groups treated “Can Huyet Vuong” and the control group.

Overall, the findings of this study indicated that no significant differences were observed in blood profile, biochemistry parameters and histopathological observations of liver and kidney tissues between the groups treated “Can Huyet Vuong” and the control group.

Our study was consistent with the previous report about the toxicity of components in “Can Huyet Vuong”. According to the study of Murugan S et al (2021), 90-day subchronic oral toxicity study, *Wistar* rats were given the administration of dose levels of 250, 500, and 1000 mg/kg of extract of Curcuma longa. The results showed that no deaths, behavioral changes, or signs of toxicity from body weight gain, food consumption, ocular and neurological examination, and hematological, blood biochemical, hormone, urine analysis or gross pathological and histopathological examination. [12] A 28-day subacute oral toxicity study was conducted to evaluate the toxicity of honey in *Wistar* rats. The results revealed that the administration at levels of 3, 6, 12, and 24 g/kg body weight/day of honey to the rats for a consecutive 28 days did not cause

significant change in clinical observations, hematological, chemical and histopathological examination as compared with the control group. [13]

5. CONCLUSION

"Can Huyet Vuong" at doses of 1.8 g/kg b.w/day and 5.4 g/kg b.w/day administered orally during continuous 30 days did not make any toxic signs or symptoms of toxicities on general indexes, hematopoietic, liver and kidney function in *Wistar* rats.

Acknowledgments

The authors would like to thank all researchers who participated in supporting this study from the Department of Pharmacology, Hanoi Medical University.

REFERENCES

1. Krishnan S. (2018). TRADITIONAL HERBAL MEDICINES - A REVIEW. **5**, 611–614.
2. WHO global report on traditional and complementary medicine 2019. <https://www.who.int/publications/i/item/978924151536>, accessed: 09/06/2024.
3. Venkatasubbu G.D., Ramasamy S., Gaddam P.R., et al. (2015). Acute and subchronic toxicity analysis of surface modified paclitaxel attached hydroxyapatite and titanium dioxide nanoparticles. *Int J Nanomedicine*, **10 Suppl 1**(**Suppl 1**), 137–148.
4. Debnath B., Singh W.S., and Manna K. (2023). A phytopharmacological review on Ananas comosus. *ADV TRADIT MED (ADTM)*,**23**(**2**),291–298.
5. Jyotirmayee B. and Mahalik G. (2022). A review on selected pharmacological activities of Curcuma longa L. *International Journal of Food Properties*, **25**(**1**), 1377–1398.
6. Lu B., Wu X., Tie X., et al. (2005). Toxicology and safety of anti-oxidant of bamboo leaves. Part 1: Acute and subchronic toxicity studies on anti-oxidant of bamboo leaves. *Food and Chemical Toxicology*, **43**(**5**), 783–792.
7. Organization W.H. (2007), *WHO guidelines for assessing quality of herbal medicines with reference to contaminants and residues*, World Health Organization.
8. World Health Organization *Working group on the safety and efficacy of herbal medicine*, Report of regional office for the western pacific of the World Health Organization; 2000.
9. Adamou A. (2022). An Overview of Subchronic and Chronic Toxicology. *Drug Discovery and Evaluation: Safety and Pharmacokinetic Assays*. Springer International Publishing, Cham, 1–14.
10. Agents C., Toxicology B., Studies D., et al. (2006), *Toxicity testing for assessment of environmental agents: Interim report*, .
11. Kalas M.A., Chavez L., Leon M., et al. (2021). Abnormal liver enzymes: A review for clinicians. *World J Hepatol*, **13**(**11**), 1688–1698.
12. Murugan S., Solanki H., Purusothaman D., et al. (2021). Safety Evaluation of Standardized Extract of Curcuma longa (NR-INF-02): A 90-Day Subchronic Oral Toxicity Study in Rats. *BioMed Research International*, **2021**.
13. Du H., Zhang P., Zheng S., et al. (2023). A 28-day subacute oral toxicity study of Apis cerana (Fabricius) honey in Wistar rats. *International Food Research Journal*, **30**, 1481–1494.