EVALUATION OF SUBCHRONIC TOXICITY OF DIABETNA CAPSULES IN EXPERIMENTAL ANIMALS

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This research aimed to evaluate the subchronic toxicity of Diabetna capsules through oral administration in experimental animals. The subchronic toxicity was studied in Wistar rats with oral doses of 0.72 g/kg/day (equal to recommended human dose) and 2.16 g/kg/day (3 times as high as recommended human dose) in consecutive 12 weeks, following guidance from the World Health Organization and Organisation for Economic Co-operation and Development. Our result showed that Diabetna capsules had no deleterious effect on hematological parameters, hepato-renal functions, macroscopic and microscopic images of the livers and kidneys of Wistar rats. In conclusion, Diabetna capsules did not produce subchronic toxicity in experimental animals.

Keywords: Diabetna capsules, subchronic toxicity, Wistar rats.

I. INTRODUCTION

The research on drugs sourced from natural origins has grown strongly. They often have advantages in cost as well as being safer for users.[1]

Toxicity studies are very important steps in drug research and development. To be used, drugs must be safe and effective. Toxicity studies on experimental animals are necessary to partially evaluate the drug's safety. In addition, depending on the type of drug, it is required to evaluate for other toxicities such as reproductive and developmental toxicity and immunotoxicity...[2] To evaluate biological toxicity, it is crucial to choose the correct system since no effect may be seen. Toxicity of a substance can be impacted by many factors, such as the route of exposure (skin absorption, ingestion, inhalation, or injection), the time of exposure (brief, acute, subchronic, or chronic exposure), the number of exposures (a single dose or multiple doses), the physical form of the toxin (solid, liquid, or gas), the organ system involved (cardiovascular, nephro-, hemo-, nervous-, or hematopoietic-system), and even the genetic makeup and robustness of the target cells or organisms.[3] Subchronic systemic toxicity is defined as adverse effects occurring after repeated or continuous administration of an investigational product for up to 12 weeks or not exceeding 10% of the animal's lifespan.[1]

Diabetna capsules were an herbal product that included *Gymnema Sylvestre* extract. *Gymnema Sylvestre* has been used for a long time, according to clinical experience, to support the treatment of diabetes and dyslipidemia.[4,5] However, up to now, there is still a lack of

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Received: 07/05/2024
Accepted: 23/05/2024

research on the safety of *Gymnema Sylvestre*. Therefore, to create a premise for studying the safety and effects of *Gymnema Sylvestre* extract in clinical, we conducted this study to evaluate the subchronic toxicity of *Gymnema Sylvestre* extract in experimental animals.

II. SUBJECTS AND METHODS

1. The investigational product

- Diabetna capsules are a herbal product prepared by Nam Duoc Pharmaceutical Company from herbal plants, including *Gymnema Sylvestre* extract.

- Each capsule contains*: Gymnema* extract 0.210 g *(*equivalent to about 1.7 g dry herbal*)*

2. Chemicals and laboratory equipment

- Kits for testing enzymes and metabolites in blood: ALT (alanine aminotransferase), AST (aspartate aminotransferase), total bilirubin, albumin, total cholesterol, creatinine kits from Erba - Germany.

- Blood-testing solutions ABX Minidil LMG of ABX Diagnostics were used for Vet abcTM Animal Blood Counter.

- Chemicals for tests and histopathological examination

3. Experimental animals

Healthy *Wistar* rats of either sex weighing 160 ± 20 g were procured from The Center of Experimental Animals, Dan Phuong, Ha Noi. The animals were individually housed in cages and fed a standard certified diet. They were acclimated to housing seven days before investigation at the laboratory of the Department of Pharmacology, Hanoi Medical University.

4. Methods

Subchronic toxicity study was carried out according to WHO Guidance and OECD guidelines.

The study was carried out in a continuous period of 12-week. *Wistar* rats were divided into three groups of ten animals (five males and 5 females)

- Group 1 (control group, n = 10): Rats were administered 1 mL distilled water/100 g b.w/day;

- Group 2 (n = 10): Rats were administered Diabetna capsules at the dose of 0.72 g/kg/day (equivalent to the human recommended dose, conversion ratio 6);

- Group 3 (n = 10): Rats were administered Diabetna capsules at the dose of 2.16 g/kg/day (3 times as high as the dose in group 2).

Animals were treated daily by oral once a day in the morning for 12 consecutive weeks and were observed once daily to detect signs of toxicity. Diabetna capsules were decapsulated, dissolved the powder inside capsules with distilled water before giving orally to rats.

The signs and indexes were checked during the study, including:

The 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 aminotransferase (ALT), total bilirubin, albumin, total cholesterol, and creatinine levels.

The parameters were checked at the following times: pre-dose, after treatment: 4 weeks, 8 weeks, and 12 weeks

At the end of the experiment, all animals were subjected to a full gross necropsy. The liver and kidney of 30% of each group's Liver and kidney of 30% rats will be removed for histopathology examinations. The micro-histological examination was conducted at the

Center for Research and Early Detection of Cancer (CREDCA), Vietnam.

5. Statistical analysis

The results were statistically analyzed using the Student's t-test and the Avant-après test. The data were expressed as the mean ± SD. All data were considered significant at $p < 0.05$.

III. RESULTS

1. General condition and body weight changes

During the experiment, rats in all three groups had normal activity, good feedings, agility, bright eyes, and dry stools.

Table 1. Effect of Diabetna capsules on the body weight change

Time	Body weight (g)($\overline{X}$ ± SD)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	157.0 ± 6.7	162.0 ± 6.3	165.0 ± 10.8	> 0.05
After 4 weeks	190.0 ± 12.5	187.0 ± 22.1	204.0 ± 23.2	> 0.05
p (pre-post)	< 0.001	< 0.01	< 0.001	
After 8 weeks	193.0 ± 18.9	188.0 ± 22.0	204.0 ± 25.0	> 0.05
p (pre-post)	< 0.001	< 0.01	< 0.001	
After 12 weeks	225.0 ± 21.7	212.0 ± 23.9	241.0 ± 27.3	> 0.05
p (pre-post)	< 0.001	< 0.001	< 0.001	

Table 1 showed that after administration of the test products for 4 weeks, 8 weeks, and 12 weeks, the body weight of the rats in all three groups increased compared to pre-dose weight ($p < 0.01$). There was no significant difference in the body weight of the rats between the treatment groups and the control group ($p > 0.05$).

2. Effect of Diabetna capsules on hemato-poietic functions

There was no significant difference in red blood cell count, hematocrit, hemoglobin level, MCV, and platelet count between groups treated with Diabetna capsules and group 1 ($p > 0.05$). The results are shown in Tables 2-5.

Table 2. Effect of Diabetna capsules on red blood cells count, hemoglobin levels in rats

Time	Red blood cells count (T/L)			Hemoglobin levels (g/dL)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	9.3 ± 1.4	9.6 ± 1.0	9.6 ± 1.6	12.7 ± 2.2	13.4 ± 1.4	13.9 ± 2.4	> 0.05
After 4 weeks	8.7 ± 1.1	9.0 ± 1.1	8.9 ± 1.2	12.0 ± 1.2	12.8 ± 1.1	12.6 ± 1.6	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 8 weeks	8.3 ± 0.7	9.2 ± 1.5	9.0 ± 0.8	11.3 ± 1.0	12.3 ± 1.2	12.2 ± 1.3	> 0.05

Time	Red blood cells count (T/L)			Hemoglobin levels (g/dL)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 12 weeks	8.2 ± 1.0	8.6 ± 1.4	8.2 ± 1.4	11.1 ± 1.1	12.2 ± 1.4	12.2 ± 1.4	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Table 3. Effect of Diabetna capsules on hematocrit and mean corpuscular volume (MCV) in rats

Time	Hematocrit (%)			MCV (fl)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	46.7 ± 7.2	48.4 ± 5.0	51.0 ± 9.0	50.1 ± 2.8	50.9 ± 1.9	51.3 ± 2.7	> 0.05
After 4 weeks	41.8 ± 4.6	43.7 ± 6.4	44.3 ± 6.3	48.5 ± 2.2	48.8 ± 2.7	50.2 ± 2.2	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 8 weeks	40.2 ± 7.3	44.1 ± 7.5	44.9 ± 3.3	48.3 ± 2.6	49.1 ± 2.4	50.0 ± 0.9	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 12 weeks	40.3 ± 8.0	41.6 ± 9.5	42.4 ± 9.6	46.7 ± 4.5	48.7 ± 2.9	49.2 ± 1.8	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Table 4. Effect of Diabetna capsules on the number of white blood cell (WBC) and platelets (PLT) in rats

Time	WBC (G/l)			PLT (G/l)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	9.8 ± 3.6	10.0 ± 2.4	9.5 ± 2.7	521.9 ± 172.9	408.1 ± 130.7	459.9 ± 181.7	> 0.05
After 4 weeks	8.6 ± 1.8	9.6 ± 2.1	7.9 ± 2.4	518.8 ± 147.8	516.1 ± 132.3	504.5 ± 151.7	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 8 weeks	11.6 ± 3.0	12.2 ± 2.9	12.5 ± 3.9	522.1 ± 155.4	421.7 ± 143.8	411.6 ± 68.2	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Time	WBC (G/l)			PLT (G/l)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
After 12 weeks	8.0 ± 1.6	8.5 ± 1.8	7.6 ± 1.2	559.1 ± 124.8	464.1 ± 97.2	472.1 ± 51.6	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Table 5. Effects of Diabetna capsules on the white blood cell differential count in rats

Time	LYM%			NEUT%			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	80.8 ± 5.3	78.3 ± 7.0	81.5 ± 6.7	7.5 ± 3.2	8.2 ± 1.9	7.7 ± 3.8	> 0.05
After 4 weeks	80.0 ± 6.4	80.5 ± 4.9	82.0 ± 4.1	6.8 ± 3.0	7.0 ± 2.7	5.6 ± 2.2	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 8 weeks	76.6 ± 4.2	75.0 ± 4.1	73.4 ± 12.4	6.6 ± 2.5	7.0 ± 2.5	7.6 ± 3.5	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 12 weeks	81.1 ± 7.2	79.7 ± 4.3	76.9 ± 3.6	6.5 ± 3.4	6.7 ± 3.2	6.7 ± 1.7	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

3. Effect of Diabetna capsules on liver functions

There were no significant differences in aspartate aminotransferase (AST) levels and alanine aminotransferase (ALT) levels, total bilirubin, albumin concentration, and total cholesterol concentration between groups treated with Diabetna capsules and group 1 (p > 0.05). The results are shown in Tables 6-8.

Table 6. Effects of Diabetna capsules on AST and ALT in the blood of rats

Time	AST (UI/l)			ALT (UI/l)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	93.2 ± 21.9	108.2 ± 24.6	102.8 ± 17.8	44.4 ± 9.4	46.3 ± 11.9	45.0 ± 11.2	> 0.05
After 4 weeks	86.5 ± 14.3	108.0 ± 29.3	94.0 ± 22.5	38.4 ± 13.9	41.0 ± 12.4	37.6 ± 11.5	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Time	AST (UI/l)			ALT (UI/l)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
After 8 weeks	74.8 ± 17.0	89.1 ± 15.1	90.8 ± 18.2	36.4 ± 13.6	39.8 ± 13.0	38.1 ± 6.4	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 12 weeks	76.3 ± 17.3	87.1 ± 21.3	83.4 ± 23.5	37.0 ± 14.1	41.7 ± 13.6	36.3 ± 13.1	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Table 7. Effects of Diabetna capsules on the albumin and cholesterol levels in the blood of rats

Time	Albumin (g/dL)			Cholesterol (mmol/l)			p (t-test Student)
	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	Group 1 (n=10)	Group 2 (n=10)	Group 3 (n=10)	
Pre-dose	3.3 ± 0.2	3.2 ± 0.4	3.3 ± 0.2	58.7 ± 6.4	57.3 ± 10.0	61.1 ± 11.8	> 0.05
After 4 weeks	3.0 ± 0.3	3.0 ± 0.2	3.1 ± 0.3	49.4 ± 14.3	53.3 ± 9.2	54.2 ± 10.6	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 8 weeks	2.9 ± 0.6	2.9 ± 0.4	2.9 ± 0.6	47.1 ± 17.9	47.0 ± 12.6	47.6 ± 18.1	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	
After 12 weeks	3.1 ± 0.2	2.8 ± 0.5	3.0 ± 0.4	48.1 ± 16.6	44.5 ± 18.6	50.3 ± 12.4	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	> 0.05	

Table 8. Effects of Diabetna capsules on the bilirubin levels in the blood of rats

Time	Bilirubin (mmol/L)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	9.6 ± 0.7	9.1 ± 0.3	9.3 ± 0.6	> 0.05
After 4 weeks	9.2 ± 0.9	8.8 ± 0.5	9.2 ± 0.5	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	
After 8 weeks	9.3 ± 0.6	8.9 ± 0.7	9.2 ± 0.8	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	
After 12 weeks	9.1 ± 1.0	8.9 ± 0.7	9.1 ± 0.4	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	

4. Effect of Diabetna capsules on kidney functions

Table 9. Effects of Diabetna capsules on the creatinine levels in the blood of rats

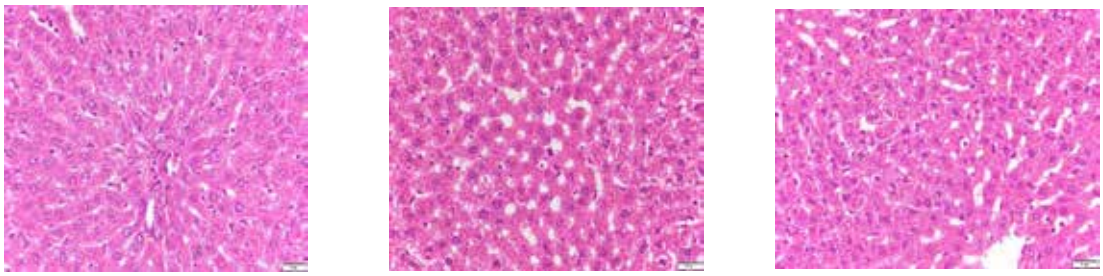
Time	Bilirubin (mmol/L)			p (t-test Student)
	Group 1 (n = 10)	Group 2 (n = 10)	Group 3 (n = 10)	
Pre-dose	70.6 ± 5.9	71.7 ± 6.2	74.0 ± 5.9	> 0.05
After 4 weeks	77.7 ± 9.0	78.3 ± 8.6	78.1 ± 8.8	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	
After 8 weeks	65.5 ± 5.1	65.7 ± 7.7	67.6 ± 8.1	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	
After 12 weeks	67.3 ± 3.7	70.5 ± 5.3	68.4 ± 6.5	> 0.05
p (pre-post)	> 0.05	> 0.05	> 0.05	

The results in Table 9 showed that after administration of the test products for 4 weeks, 8 weeks, and 12 weeks, in both treatment groups 2 and 3, the concentration of creatinine in the blood of rats did not show significant differences compared to the Group 1 (control group) and compared to pre-dose levels (p > 0.05).

5. Effects of Diabetna capsules on liver and kidney histopathology in rats

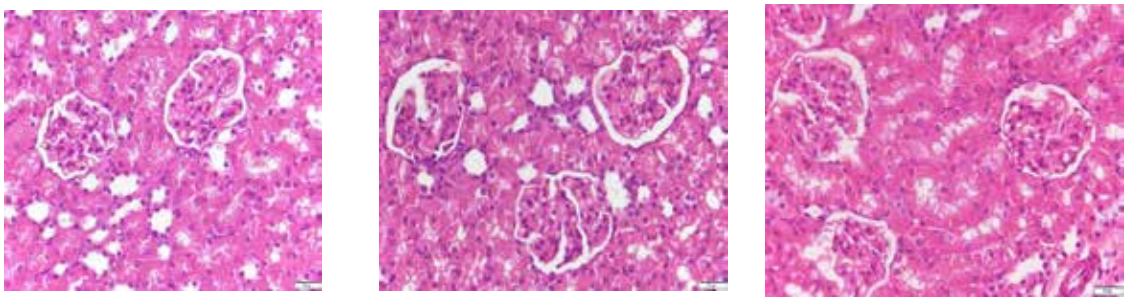
No significant gross lesions or changes were detected in liver and kidney tissues in all groups

There was no significant difference in histopathological examinations of livers and kidneys between Diabetna-treated rats and the control group after 12 weeks of treatment (Figure 1 and Figure 2)



Group 1 Group 2 Group 3

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



Group 1 Group 2 Group 2

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

IV. DISCUSSION

General condition and hematopoietic functions

According to the World Health Organization guidelines, mandatory tests include assessing the general condition, body weight, and hematopoietic functions when evaluating the toxicity of test products. Blood is very important and is closely connected to every part and organ in the body.[6] Blood is affected by all tissues and reflects the specific condition of the hematopoietic organ. If test products impact the hematopoietic organ, the blood components will be changed, decreasing white blood cells. Therefore, it is necessary to conduct tests on red blood cell count, white blood cell count, differential white blood cell count, and platelet count in rats. Hemoglobin levels provide insight into the function of red blood cells, while mean corpuscular volume reflects the characteristics of anemia. Hematocrit is the percentage between red blood cells and whole blood.[7] This index will change if the test products change the number of red blood cells or cause dehydration or water retention. These results showed that after four weeks, eight weeks, and 12 weeks of the treatment, there were no significant differences in total red blood cells, hematocrit, hemoglobin level, platelet count, total WBC count, and WBC differentials between groups treated with Diabetna capsules with the control group, so it can be concluded that the administration of Diabetna capsules did not affect the hematological profile and blood formation process.

Effect of Diabetna capsules on liver and kidney functions

Analysis of kidney and liver functions is very important in the toxicity evaluation of test products as they are both necessary for the survival of an organism. The clinical biochemistry analyses were conducted to evaluate the possible alterations in hepatic and renal functions influenced by the plant products.[8] The liver releases AST and ALT, and an elevation in plasma concentration of these enzymes indicates liver damage.[1] There was no substantial change in AST and ALT levels between the group treated with Diabetna capsules and the control group. These results indicated that Diabetna capsules had no deleterious effect on liver function. Creatinine level can be used to describe the function of the kidneys.[9] No significant differences were observed in creatinine levels between the control group and groups treated with Diabetna capsules at various doses ($p > 0.05$). Thus, Diabetna capsules did not affect the liver and kidney functions.

Effects of Diabetna capsules on liver and kidney histopathology in rats

The histopathological examination revealed the an alteration in cell structure when viewed under the light microscope. Further histological study could furnish more information regarding the hepatotoxicity and nephrotoxicity of Diabetna capsules. This study showed no significant difference in histopathological examination of the liver and kidney between groups treated with Diabetna capsules and the control group.

Our results are also consistent with the published results of studies on the safety of *Gymnema*. Raji et al. (2021) conducted toxicity studies on Wistar rats, administering *Gymnema sylvestre* extract at 100 mg/kg for 21 days. This did not adversely affect the rats' hematological, liver, or kidney function.[10] According to the overview on *Gymnema sylvestre*, its safety at the recommended dosage has been demonstrated. When *Wistar* rats were administered a 1%

Gymnema sylvestre extract mixed into their food for 52 weeks, no toxicity was detected, and no rats died during the study.[11]

V. CONCLUSION

Diabetna capsules at doses of 0.72 g/kg b.w/day and 2.16 g/kg b.w/day administered orally for 12 weeks did not produce any toxic signs or evident symptoms of subchronic toxicity in *Wistar* rats.

VI. ACKNOWLEDGMENTS

The study is funded by the project budget: "Determination of key active components with hypoglycemic effects to establish the extraction process of a standardized extract from *Gymnema Sylvestre* (Retz.) R. Br. ex Schult. and upgrade the quality standards and effects of Diabetna product". Code: 01.2021M002. The authors would like to thank Viet Nam – Korea Institute of Science and Technology and Nam Duoc Joint Stock Company for their helps and supports for this study.

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