SUBCHRONIC TOXICITY STUDY OF EFCOVIDA POWDER IN EXPERIMENTAL ANIMALS

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The purpose of this study was to evaluate the subchronic toxicity of Efcovida powder through oral administration in experimental animals. The subchronic toxicity was studied in Wistar rats with oral doses of 90 mg/kg/day and 270 mg/kg/day in 90 consecutive days based on guidance of the World Health Organization and Organisation for Economic Co-operation and Development. Our result showed that Efcovida powder had no deleterious effect on hematological parameters, hepato-renal functions, macroscopic and microscopic images of livers and kidneys of rats. In conclusion, Efcovida powder did not produce the subchronic toxicity in experimental animals.

Keywords: Efcovida powder, subchronic toxicity, experimental animals.

I. INTRODUCTION

Fullerenes, discovered in 1985 by H. W. Kroto et al., were awarded the Nobel Prize in Chemistry for this work in 1996. Fullerene molecules are composed of carbon in the form of a hollow sphere, tube, or ellipsoid. Since their discovery, the unique carbon cage structure of fullerene has gained a lot of attention in many science fields and provides immense scope for derivatization, rendering potential for various industrial applications.[1] Nowadays, fullerenes have been extensively used for many biomedical applications including the design of MRI contrast agents, X-ray imaging contrast agents, anti-HIV drugs, photodynamic therapy and targeted drug delivery systems.[2,3] Therefore, the production and usage of fullerenes are expected to escalate in the future.

However, most fullerenes are nonbiodegradable molecules whose potential toxicity has not been thoroughly investigated so far. In addition to studying the therapeutic effects, the evaluation of toxicity plays a vital role in recognizing, characterizing, and gauging their risk for human, leading to formulate measures to mitigate the risk, particularly in early clinical trials. Thus, toxicology study in experimental animals is an important step in the development of new drugs. Ingredients of Efcovida powder included Endo Fullerene, Nano Curcumine, *Glycyrrhiza uralensis*, *Ramulus Cinnamoni*, *Cordyceps militaris*, *Zingiber officinale*. The toxicity of fullerenes is, to date, poorly understood.[3] In addition, these natural products have been used since ancient times and in folklore for the treatment of many diseases and illnesses. So far, there have been no report available on the safety of a

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combination product from these components.[4] Therefore, we conducted this study to investigate the subchronic toxicity of Efcovida powder in experimental animals.

II. MATERIALS AND METHODS

1. Preparation of Efcovida powder

Efcovida powder was manufactured by Trinh Nang High-Tech Company Limited. Efcovida powder composed of Endo Fullerene 15 mg, Nano Curcumine 15 mg, *Glycyrrhiza uralensis* 15 mg, *Ramulus Cinnamoni* 15 mg, *Cordyceps militaris* 15 mg, *Zingiber officinale* 10 mg and excipients.

The expected dosage in clinical is 750 mg per day, divided into 3 doses. Efcovida powder is taken orally. The powder were dissolved with distilled water (the solvent of Efcovida powder) before giving orally for rats.

2. Experimental animals

Healthy *Wistar albino* rats of either sex, weighing 180 ± 20 g were procured from The Center of Experimental Animals, Dan Phuong, Ha Noi. The animals were acclimated to housing in the laboratory of the Department of Pharmacology, Hanoi Medical University 7 days before and during the study period. All animals maintained for 12 hours in light and dark cycle in a well ventilated house, with free access to food and water *ad libitum*.

3. Subchronic toxicity study

Subchronic oral toxicity study of Efcovida powder was performed according to the World Health Organization guideline.5,6

A total of thirty rats were divided into three groups of ten animals (five males and 5 females):

- Group 1 (control group, n=10): rats were administered orally distilled water 10 ml/kg/day.

- Group 2 (n=10): rats were administered orally Efcovida powder at the dose of 90 mg/kg/day (*equivalent to human recommended dose, conversion ratio 6*).

- Group 3 (n=10): rats were administered orally Efcovida powder at the dose of 270 mg/kg/day (*3 times as high as human recommended dose*).

Animals were given the oral administration of distilled water and Efcovida powder with the volume 10 mL/kg b.w daily for 90 days and observed once daily to detect clinical signs and time points for laboratory tests.

The signs and parameters were checked during the study:

- General conditions, body weight changes;

- Evaluation of hematopoietic function through red blood cells count, hemoglobin, hematocrit, total white blood cells (WBC), WBC differentials, platelet count;

- Evaluation of liver damage through aspartate amino transferase level (AST) and alanine amino transferase level (ALT);

- Evaluation of liver function through total bilirubin, albumin and total cholesterol;

- Evaluation of kidney function through creatinine level.

Parameters were checked before treatment, after 30 days, 60 days and 90 days of treatment. At the end of the experiment, rats were euthanized after blood collection and the internal organs (heart, liver, spleen, kidney, and lungs) were removed and observed for any gross lesions. The liver and kidneys of 30 percent of the animals of each group were preserved in 10% buffered formaldehyde solution for histopathological studies. Histopathology studies were performed at the Center for Research and Early Detection of Cancer, Vietnam.

4. Statistical analysis

The results were analyzed statistically by

Student's t-test and paired t-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, animals at all groups had normal locomotor activities, good feedings, agility, skin, fur colors, bright eyes, and dry stools. As shown in Fig.1, after 30 days, 60 days and 90 days of treatment, body weight of treated rats increased as compared with before treatment (p<0.05). There was no significant difference between the treated groups and the control group (p>0.05).

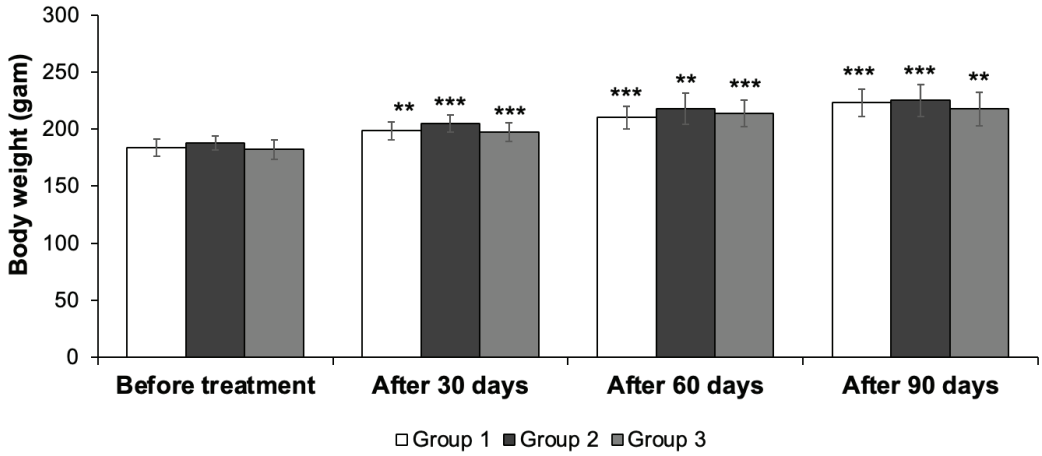


Figure 1. The effect of Efcovida powder on body weight changes

*p<0.05, **p<0.01, ***p<0.001: as compared with the time point "Before treatment"

2. Effect of Efcovida powder on hematopoietic function

There were no significant differences in red blood cells count, hematocrit, hemoglobin level, platelet count, total WBC count and WBC between Efcovida powder-treated groups and control group (p>0.05) (shown in table 2).

Table 2. The effect of Efcovida powder on hematopoietic function

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Red blood cells count (T/L)	*Group 1*	7.72 ± 0.64	8.01 ± 0.92	8.22 ± 1.00	8.00 ± 0.82
	Group 2	7.43 ± 1.05	8.01 ± 0.92	7.90 ± 1.10	7.64 ± 1.03
	Group 3	8.01 ± 0.92	8.19 ± 1.18	8.22 ± 0.89	8.36 ± 0.85
Hemoglobin level (g/dL)	*Group 1*	10.93 ± 0.92	10.35 ± 1.42	11.19 ± 0.61	10.78 ± 1.04
	Group 2	10.31 ± 0.98	10.19 ± 1.08	10.41 ± 1.16	10.50 ± 1.50
	Group 3	11.02 ± 1.13	10.93 ± 1.31	10.89 ± 1.06	10.85 ± 0.91

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Hematocrit (%)	*Group 1*	42.36 ± 3.69	42.65 ± 4.33	44.74 ± 4.19	43.71 ± 3.95
	Group 2	40.69 ± 4.02	42.17 ± 4.42	42.53 ± 4.53	40.54 ± 3.92
	Group 3	43.94 ± 3.67	43.47 ± 4.54	45.34 ± 4.47	45.17 ± 5.00
Mean corpuscular volume (fL)	*Group 1*	53.10 ± 1.52	53.00 ± 1.70	52.60 ± 2.88	52.50 ± 2.01
	Group 2	52.20 ± 2.20	52.00 ± 1.33	50.40 ± 3.03	50.90 ± 2.88
	Group 3	52.40 ± 1.07	51.90 ± 1.20	51.70 ± 1.34	52.00 ± 1.49
Platelet count (G/L)	*Group 1*	504.20 ± 84.68	554.20 ± 81.20	524.70 ± 79.65	507.20 ± 70.81
	Group 2	551.60 ± 77.78	578.60 ± 99.33	576.40 ± 79.55	548.90 ± 86.28
	Group 3	514.30 ± 94.41	526.40 ± 80.96	503.40 ± 69.29	530.60 ± 92.05
Total WBC count (G/L)	*Group 1*	8.32 ± 1.59	8.25 ± 1.37	8.13 ± 1.69	7.49 ± 1.57
	Group 2	9.03 ± 1.25	9.38 ± 1.38	8.07 ± 1.56	7.99 ± 1.35
	Group 3	7.79 ± 1.49	8.73 ± 1.43	8.53 ± 2.04	8.01 ± 1.78
Lymphocytes (%)	*Group 1*	73.84 ± 5.82	71.36 ± 6.86	74.20 ± 5.17	69.47 ± 7.30
	Group 2	71.05 ± 5.99	69.78 ± 3.92	72.96 ± 5.83	72.96 ± 5.83
	Group 3	74.92 ± 5.13	71.94 ± 4.20	73.62 ± 6.78	73.84 ± 5.81
Neutrophils (%)	*Group 1*	13.63 ± 4.13	14.93 ± 3.31	13.26 ± 3.49	14.85 ± 4.12
	Group 2	15.36 ± 3.35	17.13 ± 5.09	14.53 ± 4.30	12.59 ± 3.14
	Group 3	12.70 ± 3.06	14.37 ± 3.32	12.52 ± 3.17	11.85 ± 3.95

3. Effect of Efcovida powder on liver damage

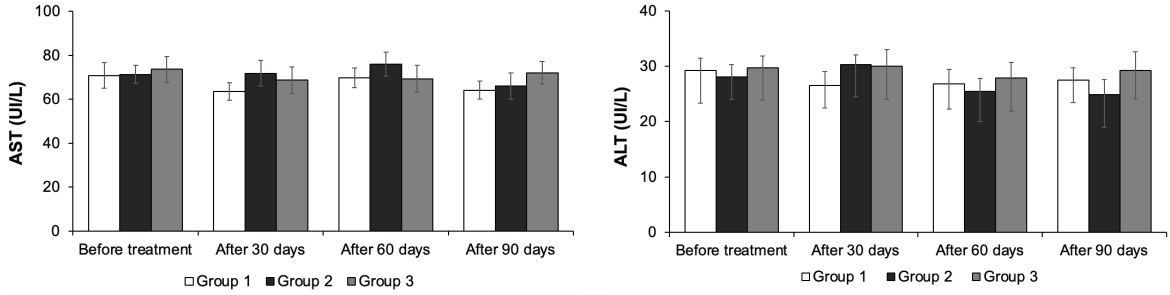


Figure 2. Effect of Efcovida powder on AST and ALT level

Fig. 2 demonstrates that after 30 days, 60 days and 90 days of treatment, Efcovida powder at doses of 90 mg/kg/day and 270 mg/kg/day did not cause statistical difference in AST and ALT level when comparing the treated groups to the control group ($p>0.05$).

4. Effect of Efcovida powder on liver function

Table 2 illustrates that after 30 days, 60 days and 90 days of treatment, there was no statistical difference in total bilirubin, albumin and total cholesterol concentration in the all treated groups when compared to the control group (p>0.05).

Table 2. The effect of Efcovida powder on on liver function

Parameters	Group	Before treatment	After treatment		
			30 days	60 days	90 days
Total bilirubin (mmol/l)	*Group 1*	13.35 ± 0.52	13.24 ± 0.27	13.46 ± 0.37	13.43 ± 0.46
	Group 2	13.09 ± 0.74	13.43 ± 0.32	13.23 ± 0.40	13.61 ± 0.44
	Group 3	13.39 ± 0.55	13.47 ± 0.33	13.43 ± 0.28	13.58 ± 0.51
Albumin concentration (g/dl)	*Group 1*	2.62 ± 0.23	2.67 ± 0.16	2.59 ± 0.14	2.52 ± 0.21
	Group 2	2.57 ± 0.25	2.73 ± 0.15	2.65 ± 0.16	2.54 ± 0.30
	Group 3	2.51 ± 0.35	2.78 ± 0.21	2.63 ± 0.20	2.68 ± 0.21
Total cholesterol concentration (mmol/l)	*Group 1*	1.29 ± 0.17	1.26 ± 0.14	1.25 ± 0.14	1.23 ± 0.19
	Group 2	1.27 ± 0.14	1.30 ± 0.13	1.31 ± 0.14	1.21 ± 0.16
	Group 3	1.30 ± 0.18	1.29 ± 0.16	1.24 ± 0.19	1.27 ± 0.18

5. Effect of Efcovida powder on kidney function

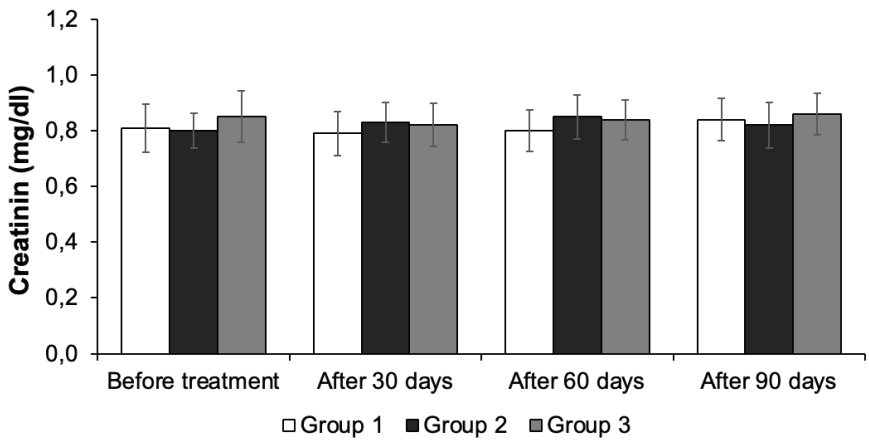
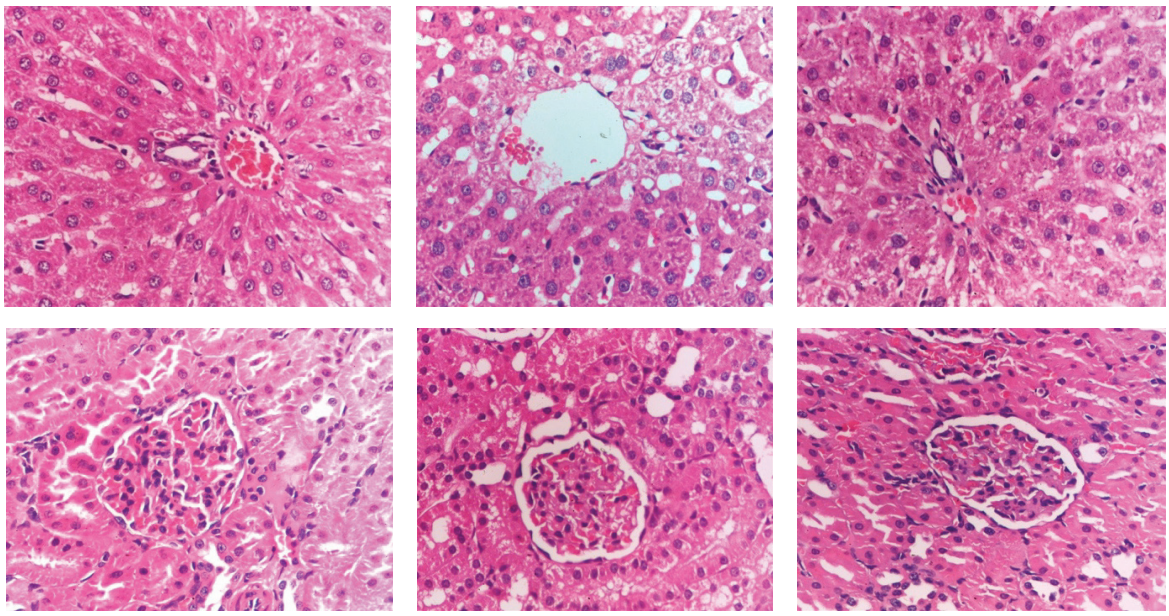


Figure 3. Effect of Efcovida powder on creatinine level

Figure 3 demonstrates that after 30 days, 60 days and 90 days of treatment, Efcovida powder at doses of 90 mg/kg/day and 270 mg/kg/day did not cause statistical difference in creatinine level when comparing the treated groups to the control group (p>0.05).

6. Histopathological examination

No gross lesions or changes in size were observed when subjected all experimental rats to a full gross necropsy which examined of the hearts, livers, lungs, kidneys and abdominal cavities. There were no significant differences in histopathological examinations of livers and kidneys between Efcovida powder treated rats and control group.



Group 1 (rat#03) | **Group 2 (rat#16)** | **Group 3 (rat#29)**

Figure 4. Histopathological morphology of liver and kidney (HE × 400)

IV. DISCUSSION

Toxicity refers to unwanted effects on biological systems. To evaluate biological toxicity, it is very important to choose the correct system, since no effects may otherwise be seen. Toxicity of a substance can be impacted by many factors, such as the route and time of exposure, the physical form of the toxin, the organ system involved.[7,8] Subchronic study provides information on the undesirable effects of continuous or repeated exposure of plant extracts or compounds over a portion of the average life span of experimental animals, such as rodents.[5,6]

The changes in body weight are the most basic index to reflect general health status of animals and also reflect the combined effects of xenobiotics on the body.[6] In our study, weight gains were observed in all animals administered with Efcovida powder. In addition, none of the Wistar animals in all treated groups showed any macroscopic or gross pathological changes when compared to the control group. It can be stated that Efcovida powder did not interfere with the normal metabolism of animals as corroborated by the non-significant difference from the control group. The blood circulatory system performs important functions. The hematopoietic system is one of the most sensitive targets of toxic compounds and is an important parameter of physiological and

pathological status in animals.[6] Furthermore, such analysis is relevant to risk evaluation as changes in the hematological system have higher predictive value for human toxicity. Our results showed that, after 90 days of treatment, there were no significantly difference in total red blood cells, hematocrit, hemoglobin level, platelet count, total WBC count and WBC differentials between the Efcovida powder-treated groups with control group. Thus, it can be concluded that the Efcovida powder have no effect on the hematological system.

Analysis of kidney and liver is very important in the toxicity evaluation of drug test as they are both necessary for the survival of an organism. The biochemistry analyses were carried out to evaluate the possible alterations in hepatic and renal functions influenced by the product.[9] Total bilirubin, albumin and total cholesterol are useful indices of the excretory function of the liver. Besides, ALT and AST are useful indices for identifying inflammation and necrosis of the liver. Accordingly, the liver releases AST, ALT and an elevation in plasma concentration is an indicator of liver damage. Moreover, creatinine levels can be used in describing the function of the kidneys.[5] In view of the serum biochemical parameters of the animals treated with Efcovida, there are no significant changes in AST and ALT in both male and female rats at all doses. Moreover, the blood biochemistry level of Efcovida powder-treated groups were presented no significantly differences when compared to the control group. These evidents show that Efcovida powder did not affect the liver and kidney functions. Furthermore, histopathological examination of the liver and kidney of the control group and all treated groups did not reveal any morphological differences. This is consistent with the results of biochemical parameters in Efcovida-treated groups.

Overall, the findings of this study indicated that no significant differences were observed in blood parameters, biochemistry parameters and histopathological observations of liver and kidney tissues between the Efcovida powder treated groups and the control group. Our study was consistent with the result from the previous reports about toxicity of each component in Efcovida powder. To date, the toxicity of fullerenes is poorly understood and contradictory in some cases. However, experimentation on fullerene toxicity testing has demonstrated in aquatic animals. No studies have conducted subchronic toxicity of fullerene on rodents. Besides, *Cinnamomi ramulus* has been applied for thousands of years in China as a relatively safe herbal medicine. Zhao et al. reported that *Cinnamomi ramulus* had no toxicity following the 30-day feeding test (5, 2.5 and 1.25 g/kg) in rats. And there were no significant pathological changes in the liver and kidney.[10] According to Chanakan Jantawong, curcumine-loaded nanocomplexes at dose of 0.09, 0.27 g/kg body weight/day for mice and 0.18, 0.54 g/kg body weight/day for hamsters did not induce any side effects in chronic toxicity tests in either animal species.[11] The data of toxicology of *Glycyrrhiza uralensis* extract demonstrated that the administration of *Glycyrrhiza uralensis* extract at doses (50, 100, 500, and 1,000 mg/kg) for 120 days did not show significantly differences at blood pressure, hematological, and biochemical parameters, and histopathology on mice.[12] Over a 13-week repeated oral dose toxicity study in *Sprague-Dawley* rats, no mortality or toxicological changes were observed up to 5000 mg/kg/day *Glycyrrhiza radix.*[13] In summary, these observations indicate that Efcovida powder did not produce subchronic toxicity in experimental animals.

V. CONCLUSION

For continuous 90 days, Efcovida powder at doses 90 mg/kg/day and 270 mg/kg/day did not make any toxic signs or evident symptoms at subchronic oral toxicity.

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