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Original Paper

Etiological Tracing Test and Drug Efficacy Test in a Case of Clinical Canine Toxic Liver Disease

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Abstract

This experiment is divided into three parts. A typical case report of canine toxic liver disease was recorded at the Huazhong Agricultural University Animal Hospital. The etiology tracing test focused on the daily life risk factors that induced the disease. The pathological model of toxic liver disease in mice was successfully established by the administration of household formaldehyde, floor cleaner and chlorine-containing disinfectant tablets. The drug efficacy test was carried out by the administration of self-made traditional Chinese medicine to the model mice, and to observe the therapeutic effect of traditional Chinese medicine prescription on the mice pathological model.

It can be concluded that the daily life risk factors should be paid enough attention to in the dog's toxic liver disease. In comparison, within households that own dogs, the toxicity of chlorine-containing disinfectant is low in the normal dilution range, and its liver damage to mice is very light during the test. However, caution should be exercised when using floor cleaners and formaldehyde. Self-made traditional Chinese medicine has a good effect on this type of liver disease, but it should also pay attention to the dose, concentration and usage cycle.

Keywords

Toxic liver disease, Etiology tracing test, Drug efficacy test, Mouse pathological model

1. Introduction

The Teaching Animal Hospital of Huazhong Agricultural University received a young border collie with vomiting and jaundice as the main symptoms. The case was confirmed as a typical clinical toxic liver disease by medical history investigation, clinical symptom diagnosis, complete blood count and serum biochemical determination, Digital Radiography examination and liver tissue pathological

biopsy techniques. The whole diagnosis and treatment process lasted about 20 days. The entire diagnostic idea of this case and the medication adjustment were determined through consultations with the veterinarians at the animal hospital. The clinical report recorded the process of diagnosis and treatment of this typical case, which witnessed that this Border Collie progress from acute, life-threatening illness with severe anorexia and jaundice to a full recovery with restored vitality, the disappearance of jaundice, and a favourable prognosis.

Through communicating with the owner about the life history, contact history and disease history of the affected dog, the possible causes of the toxic liver disease were inferred from various risk factors: residual formaldehyde from home renovation, chlorine-containing disinfectant sprayed in the community due to the epidemic situation, and household floor cleaners. Therefore, a comparative study on the source of these three pathogenic substances was conducted through mouse tests.

When tracing and recording this clinical case, the author noticed a clear distinction between Chinese and Western medicine names in the prescription. For example, the Compounds Monoammonium Glycyrrhizinate and Silymarin, both of which are single-drug ingredients extracted and refined from traditional Chinese medicine, are strictly classified as Western medicine according to the definition. In current veterinary clinical practice, most drugs used are Western medicines, with traditional Chinese medicine formulas being rarely utilized. Therefore, a test was designed and a traditional Chinese medicine formula was self-formulated after reviewing the literature, primarily aimed at liver protection, kidney nourishment, and detoxification. The efficacy of this formula in treating liver toxicity was then evaluated.

2. Case Report

2.1 Basic Information

Border Collie, female, under 1 year old, not spayed, 16.8kg, slightly elevated body temperature: 39.3 ℃ (Normal rectal temperature range for dogs: 37.5 ~ 38.5 ℃ for adults, 38.5 ~ 39 ℃ for puppies).

2.2 Clinical Symptoms

Prior to arriving at the clinic, the dog vomited multiple times, with the vomitus consisting of a yellow watery mixture (dog food); exhibited excessive thirst, drinking large amounts of water, with frequent urination; had poor appetite but normal bowel movements; showed slight lethargy, reluctance to move; had yellowing of the sclera, oral mucosa, and ear skin; dry nose; the illness had persisted for a week, with rapid weight loss over six days (from 19.5 kg to 17 kg).

2.3 Clinical Test Results

Complete blood count results of the dog showed inflammation, the number of White blood cells (WBC) and Neutrophils (NEU) increased, and Mean Platelet Volume (MPV) rose. The Canine Pancreatic Lipase Test (cPL) was negative, ruling out pancreatitis. The C-Reactive Protein (CRP) level was elevated, and urinalysis also indicated inflammation, suggesting the possibility of a bacterial urinary

tract infection, with the dog's immune system being compromised. Lateral abdominal X-ray examination showed no abnormality. B-ultrasound examination found that the dog's liver was congenital dysplasia, smaller than normal dog size, and gallbladder size was normal. Pathological examination of a liver biopsy sample showed no abnormalities, with no inflammatory cell infiltration, Kupffer cell hypertrophy, hepatic parenchymal fibrosis, or nucleolar enlargement observed. Tests for adenovirus, toxoplasmosis, and leptospirosis were all negative.

Liver biochemical indices showed Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), Total Bilirubin (TBIL), and alkaline phosphatase (ALP) Aspartate Aminotransferase (AST) and Gamma-Glutamyl Transferase (GGT) increased significantly. Indicates liver inflammation, possible cholestasis, and pancreatitis. It was diagnosed as toxic liver disease by elimination.

Table 1. Biochemical Test Results of Diseased Canine

Time	2.24	2.27	2.28	3.01	3.02	3.03	3.05	3.06	3.16
ALP(20~150 IU/L)	625	826	800	714	831	799	551	490	191
ALT(10~118 IU/L)	625	869	458	426	413	421	461	565	114
TBIL(0~5.13 umol/L)		141	254	175	72	41	33	25	0.9
AST(0~41 IU/L)		383	48	47	58	71		39	10
GGT(0~8 IU/L)		17.6	63	37	38	43	49	43	31

2.4 Treatment Plan

Ceftiofur Sodium Injection (Veterinary Use) and Dexamethasone Sodium Phosphate Injection were used for anti-inflammatory treatment. Metoclopramide Hydrochloride Injection is used to stop vomiting. Inosine Injection was continued at 1 mL/day to treat acute and chronic liver diseases. Compound Monoammonium Glycyrrhizinate Injection was given at 2 mL/day to effectively reduce the release of ALT and AST. Additionally, three 50 mg Ursodeoxycholic Acid Tablets were administered daily in a single dose to effectively treat cholestatic hepatitis. Ulinastatin Injection was administered at 4*10^4 IU/day for the treatment of pancreatitis, and Sodium Glucuronate Injection at 1 mL/day was used for liver protection and detoxification. Vitamin K1 and a B-complex vitamin injection were used to stimulate appetite. Furthermore, two 90 mg tablets of Danosh (S-Adenosylmethionine) and two 140 mg tablets of Silymarin were given in a single dose for liver protection. Enrofloxacin Injection was added to treat urinary tract bacterial infections. The dog’s food was switched to liver prescription diet to reduce the metabolic burden on the liver.

After continuous administration for 10 days and daily blood sampling to detect liver biochemical indexes, the total bilirubin continued to decrease, and the prognosis was good. On March 16, most liver indexes returned to normal.

3. Method

3.1 Experimental Animals

100 SPF-grade KM mice, male, 28 ~ 35 days old, 25 ~ 35g weight, were purchased from the Laboratory Animal Center, Huazhong Agricultural University under the License Number: SCXK (E) 2020-0019. The animals were fed ad libitum and housed in an environment with a temperature of 23 ~ 25 ℃ and relative humidity of 50% ~ 70%. Liver and blood samples of mice were taken as experimental samples. All animal procedures were approved by the Animal Ethics and Welfare Committee of Huazhong Agricultural University and the Laboratory Animal Care Committee of Hubei Province, China.

3.2 Drugs and Formulations

Formaldehyde group: 40% formaldehyde solution, purchased from Sinopharm Group Chemical Reagent Co., Ltd., batch number: 20131206; Dilution method: 1mL of 40% formaldehyde diluted to 39mL distilled water, the amount of formaldehyde is 1mg/mL.

Chlorine-containing disinfection tablet group: Kefu - DKW chlorine disinfectant tablets, production company is Shandong Jiajie Water Treatment Technology Co., Ltd, batch number: 22056J, its main ingredients are 50% trichloroisocyanuric acid and sodium dichloroisocyanuric acid; dilution method: one piece of disinfectant tablets diluted in 100mL of distilled water, with an effective chlorine content of 5 mg/mL.

Floor cleaner group: Newbark floor cleaner (mosquito repellent effect), manufactured by Brandico Limited, UK, no production batch number; Dilute concentration: Undiluted.

Self-prepared Chinese medicine formula: Bupleurum, Scutellaria, Artemisia capillaris, Gardenia, Rhubarb, Dandelion, etc., concentrated to 250 ml after decoction. Purchased from Hubei Gongshengtang Traditional Chinese Medicine Co., Ltd., production batch number is 210301. The formula was decocted according to the standard decocting process, and the final concentration of Chinese medicine content was 1mg/mL.

0.9% saline.

3.3 Intervention Method

A total of 100 mice were divided into 4 groups with 25 mice in each group.

(1) Control group: 25 mice received an oral gavage of 0.5 mL saline at 12:00 noon daily, administered as a single dose.

(2) Formaldehyde group: 25 mice received an oral gavage of 0.5 mL of 1% formaldehyde at 12:00 noon daily, administered as a single dose. Each mouse ingested 0.5 mg of formaldehyde daily, with a total concentration of approximately 16.7 mg/kg, a value below the formaldehyde LD50 (800 mg/kg).

(3) Floor Cleaner group: 25 mice received an oral gavage of 0.5 mL of undiluted floor cleaner at 12:00 noon daily, administered as a single dose.

(4) Disinfection tablet group: 25 mice received an oral gavage of 0.5 mL of diluted chlorine-containing disinfectant tablet solution at 12:00 noon daily, administered as a single dose. Each mouse ingested 5

mg of trichloroisocyanuric acid daily, with a total concentration of approximately 167 mg/kg, a value below the trichloroisocyanuric acid LD50 (750 mg/kg).

All four groups were continuously administered the respective substances for 7 days via gavage. The mice were observed for changes in mental state, jaundice, and appetite. On the 7th day, two live mice from each group (including the control group) were selected for blood collection via eyeball extraction to assess liver function. The mice were then dissected, and their liver and other internal organs were placed in formalin for preservation to prepare tissue sections for pathological sections. The collected blood samples were centrifuged at 1500 rpm for 5 minutes to obtain serum, which was sent to the Huazhong Agricultural University Hospital for testing. The specific tests included TBIL, ALT, AST, ALP, GGT, and the AST/ALT ratio.

The efficacy test for traditional Chinese medicine was conducted by randomly dividing the remaining mice from the intervention groups of the etiology tracing test into 2 subgroups. In each subgroup, one group received a daily oral gavage of 0.5 mL of the concentrated traditional Chinese medicine, while the other group served as the control and received a daily oral gavage of 0.5 mL of 0.9% saline, both administered as a single dose. In total, there were 8 groups of mice, with 4 groups receiving the concentrated traditional Chinese herbal medicine (0.5 mL/dose/day) and the other 4 groups serving as controls, receiving 0.9% saline (0.5 mL/dose/day). The treatment was continued for 7 days, during which the mice were observed for changes in mental state, jaundice, and appetite.

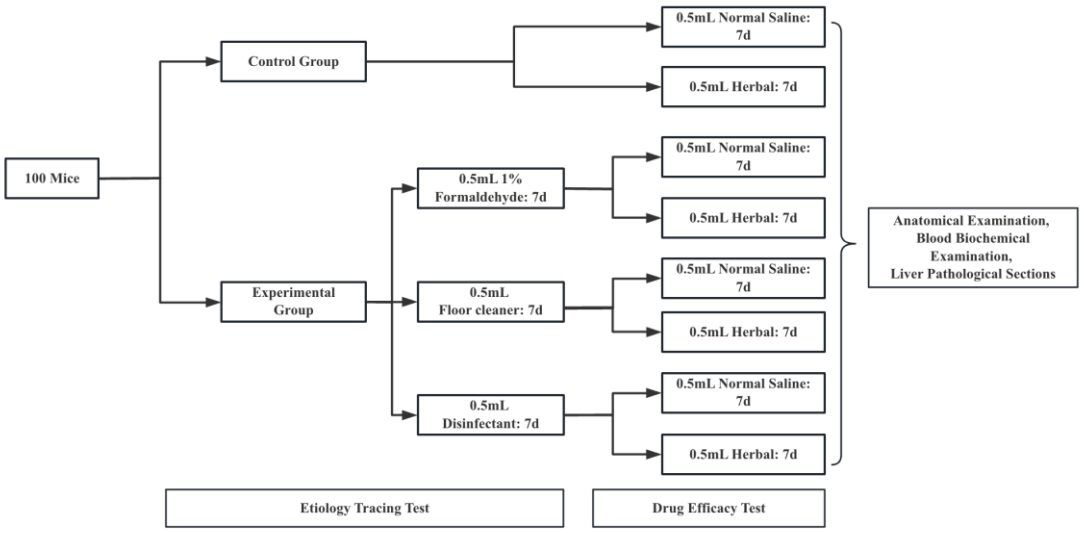

Figure 1. Grouping of Intervention Method

4. Result

4.1 Results of Etiology Tracing Test

Changes in animal behaviour state: No obvious jaundice was observed in the four groups of mice. The control group and the disinfectant tablet group had no obvious abnormal behaviour. The formaldehyde

group and the floor cleaner group had significantly reduced appetite, significantly more food remaining than the other two groups, and worse mental state, dull eyes, slow movement, about half of the mice did not want to move, low escape ability when capturing, and insensitive response; Mice in the formaldehyde group were thinner than those in the floor cleaner group. Died mice happened in all three groups, and in the formal test, the floor cleaner group had the highest number of deaths, followed by the formaldehyde group, and the chlorine disinfectant group had the lowest number of deaths; the negative control group had one death, and the autopsy death was due to improper gastric gavage, which resulted in on-the-spot death.

Ocular changes in the organs of dissected mice: at the time of sampling and dissection, the liver and lungs of the control group were bright red, and there was no abnormality in the stomach and intestines; the lungs of the floor cleaner group were reddish-white, the liver and lungs were darker, and the stomach and intestines were slightly jelly-like; the lungs of the disinfectant tablets group and the formaldehyde group were whitish, and the livers were yellowish-white, and the stomachs were slightly distended.

The results of biochemical indexes: the liver indexes of the mice in the floor cleaner group were the most abnormal when compared with the indexes of the mice in the normal control group in the experiment. This result was consistent with the behavioural changes of the mice, the results of the dissection of organs by eye, and the results of the group's mortality rate, which indicated that the liver in the floor cleaner group was generally the most seriously damaged.

Table 2. Biochemical Test Results in Four Groups of Mice after Intervention

	TBIL	ALT	AST	AST/ALT	ALP	GGT
Control Group 1	1.7	34	102	3.0	111	2
Control Group 2	1.6	32	134	4.2	112	0
Formaldehyde Group 1	0.5	32	100	3.1	90	2
Formaldehyde Group 2	0.5	35	132	3.8	97	0
Floor Cleaner Group 1	-1.5(↓)	93(↑)	320(↑)	3.4	132	1
Floor Cleaner Group 2	-0.4	44	225(↑)	5.1(↑)	106	-3(↓)
Disinfectant Tablet Group 1	1.4	75(↑)	147	2.0	242(↑)	1
Disinfectant Tablet Group 2	1.9	33	126	3.8	163	0

Pathological section results: After Hematoxylin and Eosin (HE) staining and electron microscope observation, the liver biopsy results of mice in the control group are shown in Figure A. The liver tissue appeared normal, with clear hepatic lobule structures, and no signs of hepatocyte degeneration, necrosis, or congestion in the liver sinusoids were observed. In the formaldehyde group (Figure B), no abnormalities were detected. It is speculated that this may be due to non-representative sampling,

where the samples taken did not include affected mice, and the liver tissue sections might not have included the damaged areas. For the floor cleaner group (Figure C), the liver tissue appeared pink and solid at the edges, indicating necrosis. This finding is consistent with the gross examination of the organs and the group's mortality rate, suggesting that the liver damage in this group was the most severe. This indicates that the Newbark floor cleaner used in the study has significant toxicity, and its undiluted use imposes an excessive burden on the liver. In the disinfectant tablet group (Figure D), many hepatocytes appeared as pink vacuolated structures, indicating hydropic degeneration.

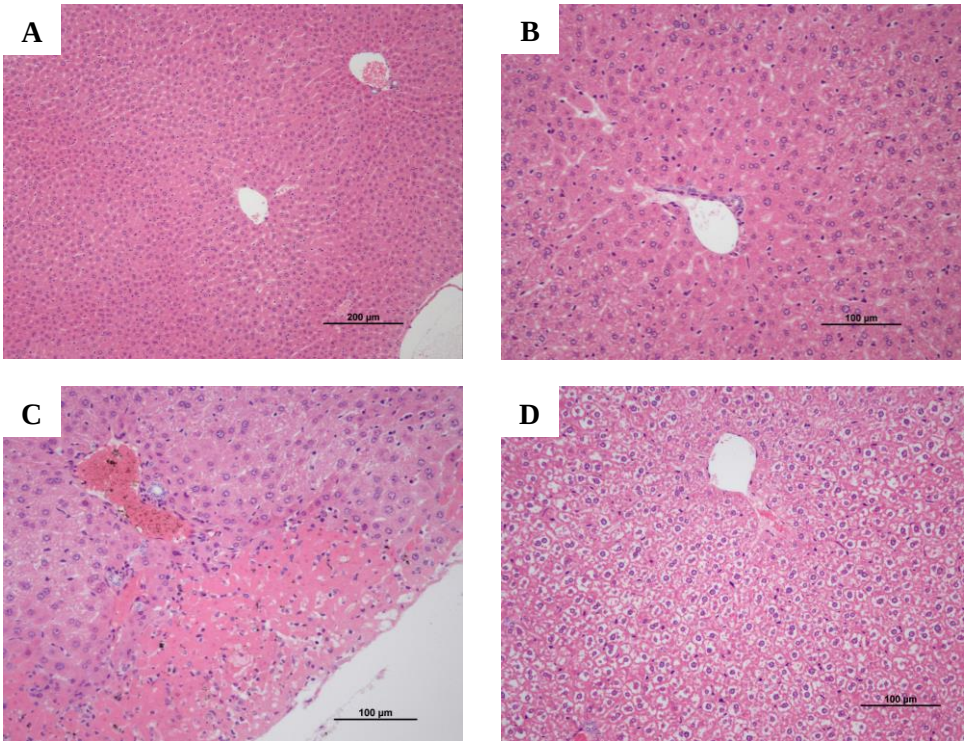

Figure 2. Liver Pathological Section Results of Four Groups of Mice after Intervention

4.2 Results of Chinese Medicine Efficacy Test

Changes in animal behaviour status: The mortality rate of mice in all groups decreased after the cessation of poisoning. However, compared with the mice given 0.9% saline by gavage, the mice in the disinfectant group and the formaldehyde group showed improvement in the state of the mice after the administration of traditional Chinese medicine by gavage, with a significantly increased appetite and more responsive, indicating that the feeding of traditional Chinese medicine could help to restore the mice's physical state and mental state. However, deaths continued to occur in the floor cleaner group, and the whole group of mice had a poor state of mind, were depressed, unwilling to walk around, were emaciated, and had the poorest appetites. The mice in the floor cleaner group continued to die. The dead mice had an enlarged abdomen, some of them had bald abdominal skin, and the skin colour was black, which showed the symptoms of chronic intoxication; the autopsy showed that the liver was

yellowish-white, with fuzzy edges, the spleen was black and red, the kidneys were a little lighter in colour, the gastrointestinal tract was filled with a lot of gases, which was jelly-like, and the lungs were whitish with pulmonary carcasses. It is suggested that the floor cleaner may cause chronic toxic disease.

Ocular changes in the organs of dissected mice: the lungs of the eight groups of mice were whitish, presumably due to the removal of the eyeballs for blood collection before the autopsy, and the ischemia led to whitish lungs; the livers and spleens of the floor cleaner group and the disinfectant group gavaged with 0.9% saline were darker than the other groups, showing a deep reddish brown; and the livers of the three groups of the poisoned group given traditional Chinese medicine by gavage were a normal reddish brown, except the liver of the chlorine disinfectant group, which showed blurred edges, with the liver of the chlorine disinfectant group showing a darker reddish brown colour. The livers of the three groups were relatively normal reddish brown, except for the disinfectant group, in which the edges of the livers were blurred. It was indicated that the administration of traditional Chinese medicine had a certain restorative effect on the livers of the poisoned mice.

The results of biochemical indexes: In the formaldehyde-herbal medicine group, the ALT and AST indexes were abnormal when compared with the indexes of the control group of mice in this experiment. The reason for this was that the water bottles in this group were empty on the day of the blood sampling, and they were not replenished in time, which led to the abnormally high indexes in the blood, and the corresponding pathological sections of this group did not show any degeneration, necrosis, or other abnormality. The indicators in the floor cleaner group, which showed the most serious liver damage after poisoning, were normal in both the Chinese medicine and saline groups by gavage. The reason for this was that the blood was taken from mice that had not died of disease and there were individual differences and the mice themselves had the function of self-recovery after stopping the poisoning, which could gradually restore their indicators to health. In addition, the high degree of concentration of traditional Chinese medicine may increase the metabolic burden of the liver and damage the liver after the mice have recovered for 7 d. This suggests that the period of drug administration is also an issue that should be paid special attention to when using drugs, or else it may cause damage to diseased animals.

Table 3. Biochemical Test Results in Eight Groups of Mice after Intervention

	TBIL	ALT	AST	AST/ALT	ALP	GGT
Control - Saline Group 1	3	60	133	2.2	87	2
Control - Saline Group 2	1.8	24	130	5.4	99	2
Control - Chinese medicine group 1	1.9	35	105	3.0	107	-1
Control - Chinese medicine group 2	3.6	33	124	3.8	109	2
Formaldehyde - Saline Group 1	2.4	28	82	2.9	140	4

Formaldehyde -Saline Group 2	3.6	26	85	3.3	129	4
Formaldehyde - Chinese Medicine Group 1	3.7	296(↑)	338(↑)	1.1(↓)	124	3
Formaldehyde - Chinese Medicine Group 2	2.8	33	103	3.1	92	0
Floor Cleaner - Saline Group 1	4.1(↑)	47	134	2.9	46	1
Floor Cleaner - Saline Group 2	3.3	33	88	2.7	88	3
Floor Cleaner - Chinese Medicine Group 1	2.0	37	154	4.2	120	0
Floor Cleaner - Chinese Medicine Group 2	2.0	28	98	3.5	76	2
Disinfectant tablets - Saline Group 1	2.9	21	92	4.4	203(↑)	2
Disinfectant tablets - Saline Group 2	2.7	29	88	3.0	137	0
Disinfection tablets - Chinese Medicine Group 1	1.6	22	74(↓)	3.4	193(↑)	4

Pathological section results: After HE staining and observation under an electron microscope, the liver pathological sections of the negative control group mice are shown in Figure 3. In both the saline group and the herbal medicine group, the liver tissue appeared normal, with clear hepatic lobule structures and no signs of hepatocyte degeneration, necrosis, or congestion in the liver sinusoids. In the formaldehyde group (Figure 4), the liver sections of mice in the saline group showed pink vacuolated hepatocytes, indicating significant hydropic degeneration. However, liver samples from mice in the herbal medicine group showed minimal degeneration, with no necrosis or other abnormalities observed. For the floor cleaner group (Figure 5), the saline group liver sections showed slight degeneration and focal necrosis with pink, solid areas. No significant lesions were observed in the liver samples from mice in the herbal medicine group. In the chlorine-containing disinfectant tablet group (Figure 6), the saline group showed hydropic degeneration and focal necrosis. However, the herbal medicine group showed no significant lesions. These results suggest that the herbal formulation can effectively protect against liver damage.

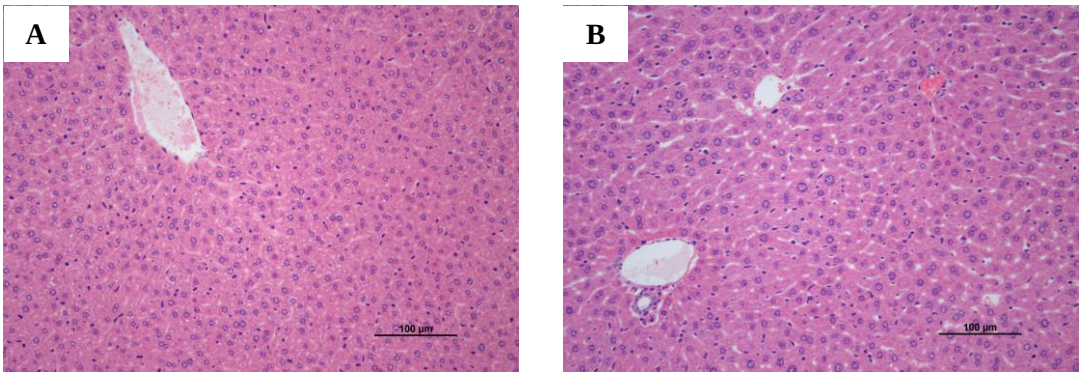

Figure 3. Liver Pathological Section Results of Control Group

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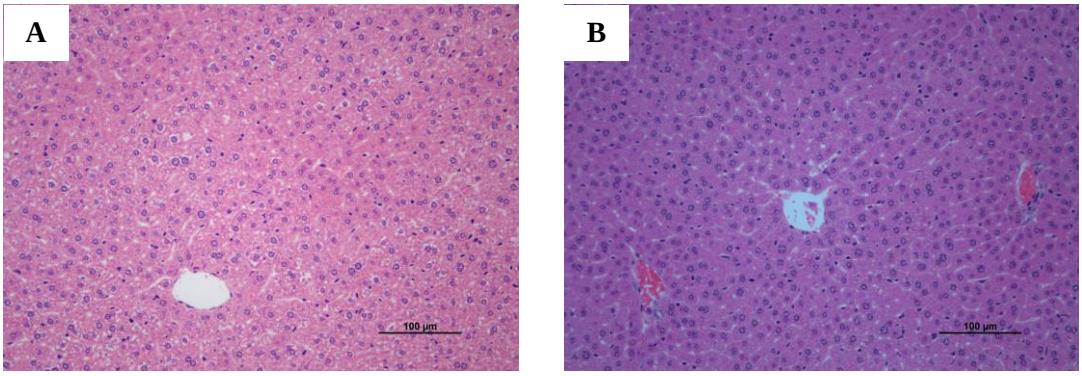

Figure 4. Liver Pathological Section Results of Formaldehyde Group

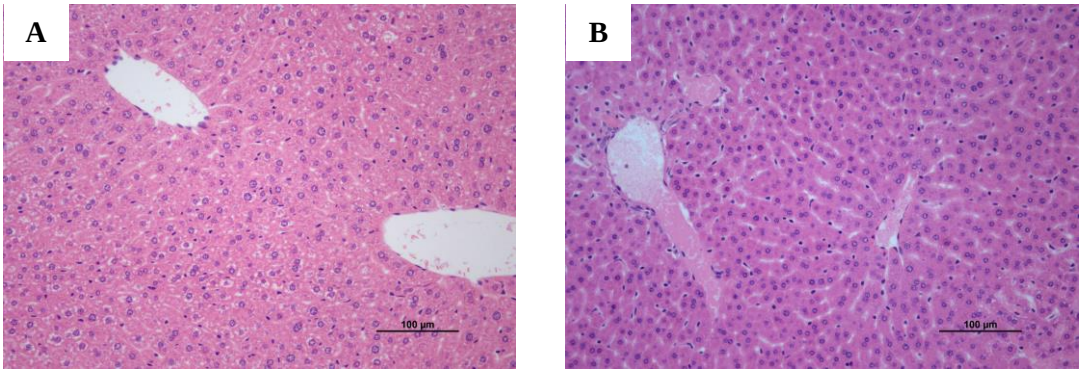

Figure 5. Liver Pathological Section Results of Floor Cleaner Group

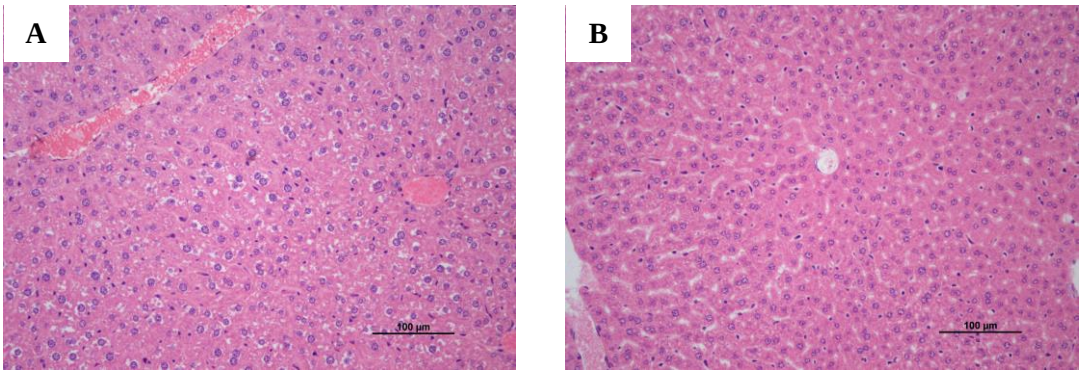

Figure 6. Liver Pathological Section Results of Chlorine-Containing Disinfection Tablet Group

5. Discussion

A 3d pre-test was conducted before the formal start of the traceability test. In the pre-test stage, the three groups of experimental groups all had different degrees of death, among which the formaldehyde group had the highest number of deaths, followed by the floor cleaner group, and the chlorine-containing disinfection tablet group had the least number of deaths. However, during the formal trial, after reducing the formaldehyde concentration, the death rate of the formaldehyde group was reduced, and the floor cleaner group had the highest number of deaths. After the autopsy of the

dead mice, the gastrointestinal tract of the mice in the floor cleaner group was either jelly-like, or seriously eroded, and the colour became dark brown or even black. This result was also consistent with the results of the pathological section and liver biochemical indexes, indicating that the liver damage in the floor cleaner group was the most serious. Therefore, it is reasonable to assume that it comes from the United Kingdom, the specific ingredients are unknown, and the ingredient list is only marked with: Water, coconut oil, citric acid, lightener, citronella extract, mugwort leaf extract, known as mosquito repellent without harm and pollution-free, Newbark floor cleaner may actually be more toxic, undiluted use will cause excessive burden on the animal liver, and not only will have toxic effects on the liver, if accidentally drunk, the floor cleaner will react with the gastrointestinal tract. Produces a large amount of corrosive substances to gastrointestinal erosion and even perforation. Questions remain as to which specific ingredient in the floor cleaner could be so toxic. After reviewing a large number of literatures, the initial suspicion is that the extract of mugwort leaves can cause liver toxicity; Citric acid may cause increased stomach acid secretion, abdominal pain, and decreased appetite.

Through communication with the owner of the affected dog, it was understood that when the floor cleaner was only used for two days without dilution, the affected dog may only lick the ground that has just been milled and drink a small amount of the floor cleaner residue, the dose should not be large, but it has a large metabolic burden on the liver of the affected dog, and even cause disease. To warn you pet owners and readers at home or with children, to carefully choose and buy disinfection drugs used at home, especially foreign disinfectant or detergent brands with unknown ingredients, there may be hidden dangers.

In contrast, with another chlorine-containing disinfection tablet sprayed during disinfection during the epidemic in the community, the concentration used in the test increased to 5 to 10 times the normal concentration, but the death rate was still the lowest of the three groups, the pathological section results showed that the liver damage was also the least, the biochemical test results were also relatively normal, indicating that such disinfection tablets were less toxic in life.

In the test of Chinese medicine efficacy, the biochemical index of mice was not ideal, and the index of mice in the formaldehyde-Chinese medicine group 1 was abnormal. The indexes of mice in other infected groups also gradually returned to normal, indicating that the body of mice had a self-healing function after stopping poisoning. However, the four examination results of mental state observation, anatomical eye view, mortality and liver pathological section of mice still showed that this Chinese medicine prescription could effectively protect liver damage, and restore appetite and mental state. But at the same time, it should also be noted that the concentration of drugs should not be too high, and the clinical drug cycle of animals should not be too long, otherwise, it will cause new burdens and damage to animals.

6. Conclusion

It can be concluded that environmental life factors should be paid enough attention to in dogs with

toxic hepatitis. In comparison, in the daily life of dog families, chlorine-containing disinfectants are less toxic after dilution of normal concentration, and floor cleaners and formaldehyde should be used with caution. The self-made Chinese medicine prescription has a good therapeutic effect on this type of liver disease, but it is necessary to pay special attention to the dose, concentration and use cycle of concentrated and tortured Chinese medicine prescription in clinical practice. If the use cycle is too long, it will cause burden and even damage to the liver.

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