
A Preliminary Study of Acute Toxicity and Genetic Toxicity of Cooking Oil Used in a Street Food Stall

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ABSTRACT

Objective The purpose of this study was to perform a preliminary toxicological evaluation on the safety of cooking oil used in street food stalls. **Methods** Based on the "Hygiene Standards of Food" (2003), the oral acute toxicity test, the AMES test, the bone marrow cell micronucleus test and the sperm abnormality test in mice were conducted to evaluate the acute and genetic toxicity of cooking oil used by a street food stall. **Results** The acute oral LD₅₀ of the cooking oil was > 15000 mg/kg. Under the condition with or without S₉, the AMES test showed that the cooking oil induced the reverse mutation in both the TA98 and TA102 strains with a significant dose-response relationship in both cases. The bone marrow cell micronucleus test and sperm abnormality test in mice showed that the rates of micronucleus and sperm deformity in each experimental group were higher than those in the negative control group with a dose-response relationship ($p < 0.05$). **Conclusion** The acute oral toxicity of the cooking oil is at a non-toxic level. The cooking oil can induce a reverse mutation in TA98 and TA102, and it can cause bone marrow micronucleus and sperm abnormality in mice, thereby suggesting that the cooking oil may have a certain genetic toxicity.

KEYWORDS: hogwash fat, Acute toxicity, Bone marrow micronucleus, Sperm abnormality

INTRODUCTION

Sidewalk snack booth is a type of street food stall, which originally referred to a restaurant with the license for outdoor cooked food in Hong Kong, and it now generally refers to the Chinese open food shops mainly selling barbecue, spicy hot pot, and skewers. The food in these stalls is mostly exposed and lacks refrigeration, and the measures used against flies and the oil used in these stalls are usually of poor quality. Moreover, some of the oil used is hogwash oil (illegal cooking oil).

Hogwash oil is a generic term for all types of inferior edible oils^[1] of which the main component is the waste oil generated in people's daily lives belonging to the category of kitchen waste. In many countries, hogwash oil is mainly used as the raw industrial material for the process of diesel refining and the production of rubber, soap and cosmetics. In China, however due to a variety of reasons, these waste oils may be used as cooking oil after a simple extraction process. The current annual consumption of hogwash oil for cooking is approximately 2 million tons, accounting for one-tenth of the total annual consumption of edible oil^[2]. Long-term consumption of hogwash oil may cause serious harm to human health due to its essential characteristics of "waste". To date, extensive research has been performed on the identification of hogwash oil.

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resulting in a number of effective detection methods [3-8]. However, with respect to the hazards of hogwash oil, the scientific and systematic findings based on animal experiments are still lacking. Therefore, in accordance with "The procedures and methods for the evaluation of food safety in toxicology" (GB15193- 2003), we performed a preliminary toxicological test for the safety of oil kindly provided by an industrial feedstock processing plant, which was used as cooking oil by a street food stall.

1. MATERIALS AND METHODS

1.1. Test substance

The cooking oil for a street food stall was provided by an industrial feedstock processing plant. The oil sample was a gray-brown semi-solid and was melted in a 100 °C water bath for an extended period of time. The oil sample had a stench, and it was suspended with peanut oil in different concentrations for the experiment.

1.2. Chemicals

Acetylsalicylic acid (99%) was purchased from Alfa Aesar and used as the positive agent for the rat teratogenicity study. Cyclophosphamide (CP) was purchased from Sigma, and mitomycin C (MMC) was purchased from Roche. 2-Aminofluorene (2-AF) was purchased from Fluka, and 1,8-dihydroxyanthraquinone (diaquone) was purchased from Aldrich.

1.3. Animals

The SPF grade healthy NIH mice or KM mice were provided by the Experimental Animal Center of Guangdong Province (Animal Production License No.: SCXK (Guangdong) 2008-0002). The animals were fed in a SPF grade animal facility (Animal feed license No.: SYXK (Guangdong) 2008-0011) under a 12 h light-dark cycle with the room temperature of 20-25°C and humidity of 40-70%. The animals were kept in plastic boxes with food and water *ad libitum*. The study protocol was approved by the Ethics Committee for Animal Care of Guangdong Provincial Center for Disease Control and Prevention.

1.4. Experimental methods

1.4.1. Oral acute toxicity test

A total of 20 SPF KM mice (10 males and 10 females) weighing 18.0-22.0 g were selected for the test. After fasting overnight, the animals received a gavage dose of 15000 mg/kg b.w. with a gavage capacity of 0.2 ml/10 g b.w., and the poisoning symptoms and death of the animals were observed and recorded daily for two weeks after administration.

1.4.2. Genetic toxicity test

1.4.2.1. AMES test

The AMES test was performed on the TA97, TA98, TA100 and TA102 standard strains of *Salmonella typhimurium* using the conventional plate incorporation method. The doses of 40, 200, 1000, 5000 µg/plate were tested with or without the presence of S9. A spontaneous revertant group, a solvent control group and a positive control group were set up at the same time.

1.4.2.2. Bone marrow cell micronucleus test

The KM mice (6-8 weeks old and weights of 25-30 g; five females and five males in each group) were assigned to groups that received 2.5, 5.0 and 10.0 g/kg b.w. oil sample doses as well as a negative control group and a positive control group (CP 50 mg/kg b.w.). The different doses were administered to the animals by gavage in a volume of 20 mL/kg b.w. The treatment was conducted twice in a 24 h interval. Sternum bone marrow smears were performed 6 h after the last treatment and stained with Giemsa. A total of 1000 polychromatic erythrocytes (PCEs) were observed in each mouse. The number of polychromatic red cells containing

micronuclei (MN) was counted to calculate the rate of micronucleus cells (%) in each group. The nuclear division index was calculated by analysis of the PCE/(PCE+NCE) ratio to determine the cytotoxicity.

1.4.2.3. Sperm abnormality test

Male NIH mice (weights of 25-35 g and approximately 7-9 weeks old) were used in the sperm abnormality test. The following five experimental groups were set up: 2.50, 5.00 and 10.00 mg/kg b.w. oil sample groups; a negative control group; and a positive control group (cyclophosphamide, 50 mg/kg). Each experimental group consisted of five animals. Different doses were administered to the animals for 5 d by gavage in a volume of 20 ml/kg b.w. The bilateral epididymis was removed for smearing and staining in Eosin on the 35th day after the first treatment. From each mouse, 1000 sperm were observed, and the number and type of malformed sperm were recorded to calculate the sperm abnormality rate (%).

1.5. Statistical analysis

For the measurement data, all experimental results were statistically analyzed by one-way analysis of variance (ANOVA). The Chi-square test, Fisher's exact test, Poisson distribution test or Wilcoxon rank sum test was used to analyze the count data. A significant difference was defined as $p < 0.05$.

2. RESULTS

2.1. Oral acute toxicity test in mice

After intragastric administration, only some mice showed reduced activity, unkempt hair, and minced feces, but most mice had no obvious toxic reactions. All symptoms of poisoning in mice disappeared after the second day, with no death of mice throughout the observation period. The test animals were sacrificed after the end of the trial. No obvious abnormalities in gross anatomy were observed. The oral acute LD50 values of hogwash oil for both male and female SPF KM mice were greater than 15000 mg/kg. According to the grading standards of toxic substances in the "Hygiene Standards of Food" (2003), the acute toxicity of this oil sample was graded at a non-toxic level.

2.2. Genetic toxicity test

2.2.1. AMES test

The hogwash oil was tested on four bacterial strains, including *Salmonella typhimurium* TA97, TA98, TA100 and TA102. With or without the presence of S9, the number of revertant colonies in the 5000, 1000, and 200 $\mu\text{g}/\text{plate}$ dose groups exceeded the spontaneous revertant colony number by 2-fold, and there was a dose-response relationship between the dosages. As shown in Table 1, the revertant colony numbers in the positive control groups were significantly higher than those in the spontaneous control groups.

2.2.2. Bone marrow cell micronucleus test in mice

Figure 1 shows a polychromatic erythrocyte with a micronucleus in mouse bone marrow. The growth and activity of the animals in each group during the trial were good with no obvious symptoms of poisoning. As shown in Table 2, the micronucleus rates of the male mice in each dose group of hogwash oil were higher than that in the negative control group, and the differences were statistically significant. The 2500 mg/kg dose, 5000 mg/kg dose ($p < 0.05$), and 10000 mg/kg dose ($p < 0.01$) groups also showed a dose-response effect in the micronucleus rates. Although the micronucleus rates of the female mice in each dose group were also higher than that in the negative control group, only the difference of the group with 10000 mg/kg dose was statistically significant ($p < 0.01$).

2.2.3. Sperm abnormality test in mice

During the trial, the growth and activity of the animals in each group were good. No obvious symptoms of poisoning were noted. The rates of sperm abnormality for the mice in each dose group were significantly higher than that in the negative control group with a statistically significant difference ($p<0.01$), and the rates of sperm abnormality for the mice in each dose group showed a significant dose-response relationship. The results of this test showed that the oil sample could cause malformation of mouse sperm (Table 3). The comparison of the composition of different types of abnormalities in each group showed that the incidences of fat head and banana-shaped deformities in each dose group of hogwash oil were significantly higher than those in the positive control group, while the incidences of amorphous and pin-shaped deformities in each dose group of hogwash oil were lower than those in the positive control group. Furthermore, the constituent ratio of no hook deformity in each dose group of hogwash oil was lower than that of the positive control. Overall, these results suggest that the hogwash oil may influence the sperm head.



Figure 1. A polychromatic erythrocyte with the micronucleus in mouse bone marrow

Table 1. Results of AMES test

Dose µg/dish	TA97		TA98		TA100		TA102	
	-S9	+S9	-S9	+S9	-S9	+S9	-S9	+S9
40	158. ± 3. 7	158. ± 1. 7	37.0 ± 0.0	34.3 ± 1.2	181. ± 6. 7	176. ± 7. 2	292.0 ± 5.1	292.3 ± 4.5
200	158. ± 5. 3	159. ± 4. 3	69.0* ± 4.1	70.3* ± 5.6	181. ± 5. 0	183. ± 4. 7	588.0* ± 17. 7	592.0* ± 15. 0
1000	159. ± 5. 7	158. ± 4. 3	137.7 ± 6.9 *	148.7 ± 12. *	182. ± 7. 1	175. ± 3. 7	886.0* ± 34. 4	893.0* ± 38. 0
5000	163. ± 3. 0	164. ± 5. 7	255.7 ± 15. *	271.3 ± 18. *	177. ± 3. 7	181. ± 5. 2	1470.0 ± 32. *	1428.3 ± 16. 5
Negative control	157. ± 7. 3	157. ± 8. 7	34.3 ± 1.2	36.3 ± 1.2	177. ± 3. 7	180. ± 4. 7	291.7 ± 8.2	293.0 ± 5.7
Solvent control	153. ± 2. 7	156. ± 4. 0	34.3 ± 0.5	34.3 ± 0.9	179. ± 5. 0	183. ± 5. 3	297.0 ± 5.7	306.7 ± 4.7
Positive control	>1000	>1000	>1000	>1000	>1000	>1000	>1000	>1000

Table 2. Results of the bone marrow micronucleus test in mice (n=5, $\bar{x}\pm s$)

Group	The number of polychromatic erythrocytes	The micronucleus rate of the male mice (‰)	The micronucleus rate of the female mice (‰)
Negative control	5000	5.00±1.00	5.40±0.93
2500 mg/kg	5000	9.80±2.28 ^a	8.40±1.33
5000 mg/kg	5000	11.6±3.21 ^a	8.60±1.33
10000 mg/kg	5000	16.6±3.78 ^b	15.2±1.56 ^b
Positive control	5000	44.8±12.44 ^b	39.6±9.99 ^b

Note: ^a indicates $p<0.05$ compared to the same gender in the negative control group, ^b indicates $p<0.01$ compared to the same gender in the negative control group.

Table 3. Impact of hogwash oil on the incidence of abnormal sperm and the constituent ratio of various types of sperm abnormality (n=5)

Group (mg/kg)	The number of abnormal sperms	The rate of sperm abnormality (x±s, %)	No hook		Fat head		Banana-shaped		Amorphous		Pin-shaped		Double heads /double tails	
			The number of sperms	Constituent ratio %	The number of sperms	Constituent ratio %	The number of sperms	Constituent ratio %	The number of sperms	Constituent ratio %	The number of sperms	Constituent ratio %	The number of sperms	Constituent ratio %
Negative control	98	1.96±2.70	24	24.49	13	13.27	22	22.45	19	19.39	20	20.41	0	0.00
2500	167	3.34±2.88 a	39	23.35	39	23.35	25	14.97	29	17.37	34	20.36	1	0.60
5000	233	4.66±3.54 a	61	26.18	45	19.31	74	31.76	21	9.01	28	12.02	4	1.72
10000	304	6.08±2.55 b	92	30.26	49	16.12	96	31.58	34	11.18	29	9.54	4	1.32
Positive control	329	6.58±6.19 b	109	33.13	60	18.24	88	26.75	37	11.25	32	9.73	3	0.91

Note: ^a indicates $p < 0.05$ compared to the negative control group, ^b indicates $p < 0.01$ compared to the negative control group.

3. DISCUSSION

Hogwash oil is also known as swill oil or illegal cooking oil, and it has the following three main sources [1]: 1) traditional hogwash oil, which is the oil from leftovers (swill) of restaurants and hotels or the floating greasy debris from the sewer after a simple refining process; 2) fried denatured oil, which is the edible oil after repeatedly frying and not passing the health standards; and 3) new hogwash oil, which is the oil extracted from poor quality, outdated, and corrupt animal skins, meat, and offal by a simple process. Hogwash oil poses many risks to health [9-11]. First, the defect of the nutritional value is a risk factor. The environment and the microbial contamination of hogwash oil cause the rapid oxidation and rancidity of fats, thus resulting in its acid value and peroxide value exceeding the standards, which will not only severely damage vitamins A, D, and E in the oil but will also damage vitamin B intake from other foods at the same time. Long-term consumption of these deteriorated fats may cause the phenomenon of poisoning and the deficiency of fat-soluble vitamins and riboflavin due to the lack of essential fatty acids. In contrast, after a large number of peroxide lipids contained in rancid grease enter the body, the peroxide lipids can easily attack cell membranes and enzymes leading to a series of chain reactions producing other harmful substances, such as free radicals, which destroy human cell membranes, de-activate serum anti-proteases, damage genes leading to mutations, and accumulate inducing cancer, atherosclerosis, cell aging and other diseases. Second, excessive cholesterol in hogwash oil is another risk factor [12]. Third, a variety of toxic and hazardous pollutants in hogwash oil exceed the standards. Due to the bad sources, simple/rough processing technology, and cross-contamination in packaging and transportation, hogwash oil is greatly polluted with chemicals and organic solvents, such as detergent, alkanes, cycloalkanes, alkenes, and aromatics. The contents of heavy metals, namely lead (Pb), total arsenic (As), mercury (Hg) and cadmium (Cd), in hogwash oil far exceeds the corresponding safety standards. Moreover, hogwash oil also contains a certain amount of carcinogenic factors, such as aflatoxin B1 and benzopyrene. Long-term repeated use of this hogwash oil will cause a variety of intoxications and even cancer. Fourth, the harm caused by repeated use of oil is also a risk factor. Hogwash oil is repeatedly heated in use, resulting in a darkened color of the oil, and many phospholipids are precipitated with increased viscosity producing a large amount of harmful substances and generating various forms of polymers, which can be absorbed by the body causing growth retardation, hepatomegaly, and fertility disorders.

The bone marrow cell micronucleus test in mammals is used to detect the injury effect of clastogen and aneuploidy-inducing agents on chromosomes. The formation of a micronucleus is a genetic end point after a cell

is affected by a genotoxicant. The increase in the rate of micronucleus formation reflects the genetic effects of chromosomal damage. Currently, the micronucleus test has been recognized as one of the most rapid and sensitive conventional methods used to detect damage in genetic material and the toxicity of chemicals in mammalian cells [13]. Sperm morphology is an indirect indicator used to evaluate the potential adverse effects of a chemical on the genetic material of sperm. Although a small number of abnormal sperm exists in semen even under normal circumstances, the overall quality of sperm is not affected. A reproductive toxicant may greatly increase the number of abnormal sperm. Thus, the level of deformity rate may reflect the reproductive toxicity of this chemical toxicant and the potential mutagenicity of germ cells [14]. To better illustrate the hazards of the ingredients in hogwash oil, the cooking oil from a street food stall provided by an industrial feedstock processing plant was selected as the oil sample for the experiments in this study. The results showed that the cooking oil had some toxicity. However, according to national standards, the oral acute toxicity LD50 value of this oil was > 15000 mg/kg, which is considered to be a non-toxic level. Compared to the control animals, the mice in each dose group showed reduced activity with loose greasy dull hair and minced feces, indicating that hogwash oil may have a certain impact on the health of the body. The results of the AMES test, the bone marrow cell micronucleus test and the sperm abnormality test in mice were all positive, suggesting that this oil sample had a certain mutagenic effect, that is, this oil sample had a certain genetic toxicity. The results of this study were significantly higher than the findings for swill oil reported by Ran Li *et al* [15], thus suggesting that even though the refining process of hogwash oil is simple, it can still remove a considerable amount of toxic ingredients to a certain extent. Further research should be performed in terms of the physical and chemical analyses of different oil samples to clarify the type and quantity of the main toxic and harmful substances in different sources of feedstock raw oil, hogwash oil and swill oil. Future studies with the combination of computer modeling and structure-activity relationship analysis should also be performed.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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REFERENCES

- [1] Yun Wuxin. The outlet and identification of hogwash oil [J]. *Life and disasters*, 2011 (10):21-22.
- [2] Huang Wei, Zheng Jianjun, Xu Jianhua. Study on safe and rapid detection of discard-oil [J]. *Journal of Shandong University of Science and Technology: Natural Science*, 2010, 29 (3):51-53.
- [3] Zhang Qing, Shen Qun. A review of the detection technology for hogwash oil in edible vegetable oils [J]. *Food Science and Technology*, 2010, 35 (10):311-314.
- [4] Cao Wenming, Xue Bin, Yang Botao, *et al.* The development and research of the detection technology for

- hogwash oil [J]. *Grain Science and Technology and Economy*, 2011, 36 (1):41-44.
- [5] Zhang Liyan, Zhou Chaojun, et al. Detection method of hogwash oil [J]. *Journal of Longyan University*, 2012, 30 (5):20-23.
- [6] Xie Mengyuan, Zhang Jun et al. Discrimination of waste cooking oil by near infrared spectroscopy [J] *China oils and fats* 2011, 36 (12):80-83.
- [7] Zhang Zhong, Ren Fei, Zhang Pan. Determination of capsaicinoids and eugenol in waste edible oil by liquid-liquid extraction and liquid chromatography-tandem mass spectrometry [J]. *Chinese Journal of Chromatography*, 2012, 30 (11):1108-1112.
- [8] Zhang Zhong, Wang Lichun, Lu Yuntian. Determination of sodium and chloride in hogwash oil and their molar ratio by ion chromatography [J] *Chinese Journal of Chromatography*, 2012, 30 (11):1113-1116.
- [9] King IB, Kristal AR, Schaffer S, et al. Serum trans fatty acids are associated with risk of Prostate cancer in beta-carotene and retinol efficacy trial. *Cancer [9] Epidemiol Biomarkers Prev*, 2005, 14:988-992.
- [10] Hajimahmoodi M, Vander Heyden Y, Sadeghi N, et al. Gas-chromatographic fatty-acid fingerprints and partial least squares modeling as a basis for the simultaneous determination of edible oil mixtures [J]. *Talanta*, 2005, 66:1108-1116.
- [11] Wang Le, Liu Yaogang, Chen Fengfei, et al. Study of the contamination and deterioration of hogwash oil. *Journal of Wuhan Polytechnic University*, 2007, 36(4):1-4, 12.
- [12] Zhang Rui, Zu Liya, Fan Tie, et al. Distinguishing hogwash oil in edible vegetable oil by determination of cholesterol content [J]. *China oils and fats*, 2006, 31(5):65-67.
- [13] Chen Jinjun, Yu Zengjie. Bone-marrow micronucleus test of extract from *Senecio scandens* Buch-Ham in mice [J]. *Chinese Journal of Traditional Veterinary Science*, 2007 (1):20-22.
- [14] Peng Shuxin, Li Xiaohong. Study of genetic toxicology for *Ioranthus parasiticus* [J]. *Chinese Journal of Birth Health & Heredity*, 2008, 16 (12):46-48.
- [15] Ran Li, Zhou Yong, Zhu Jundong, et al. Acute toxicity and genetic toxicity of hogwash fat [J]. *Journal of Third Military Medical University*, 2011, 33 (13):1366-1369.