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Subchronic Toxicity of Liquid Smoke from *'Tian Op'* in Wistar Rats

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ABSTRACT

Liquid smoke (LS) from *'Tian Op '* has been developed for 'feathering' (softening) the odor of many Thai desserts. The benefits of liquid smoke are reduced smoking time, increased ease of utilization and standardization of the intensity of the flavor in dessert products. The Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand conducted a 90 d oral subchronic toxicity test of LS in 120 Wistar rats divided into five groups (12 per sex per group). Groups 1 and 2 were control groups receiving distilled water and propylene glycol at a volume of 10 $mL.kg^{-1}.day^{-1}$, respectively, where the weight was based on live body weight. Groups 3–5 were experimental groups receiving LS at doses of 0.04, 0.4 and 4.0 $g.kg^{-1}.day^{-1}$, respectively. LS at any dosage did not affect growth, food consumption and hematological values. Rats receiving LS at doses of 0.4 and 4.0 $mL.kg^{-1}.day^{-1}$ had significantly higher albumin levels than the water control and the propylene glycol-treated rats, but these alterations did not indicate any abnormalities. Histopathology of organs revealed no abnormalities related to liquid smoke toxicity. Therefore, it may be concluded that liquid smoke at the given dosages did not produce toxicity in Wistar rats.

Keywords: liquid smoke, subchronic toxicity

INTRODUCTION

The most important and unique characteristic of Thai desserts is their flavor and appearance. Jasmine (*Jusminum adenophyllum*), Pandanus palm (*Pandanus amaryllifolius* Roxb.) and '*Tian Op* ' are widely used to enhance and 'feather' (soften) the odor of many Thai desserts. '*Tian Op* ' is well known in traditional scented candles in Thailand and has been used for fumigation. The main ingredients of '*Tian Op* ' are beeswax and other ingredients such as benzoin (*Styrax benzoin* Dryand), kaffir lime peel (*Citrus hystrix* DC.), sandalwood (*Santalum album*) and Borneo camphor (*Pogostemon cablin* (Blanco) Benth). Various fumigation methods are famous in Thailand for increasing the aroma of smoked desserts. '*num dok mai*' and '*gleep lum duan*' are two types of traditional desserts that are flavored using the '*Tian Op* ' smoking process (Watcharananun *et al.*, 2009). At the present time, there is little available information on the extent to

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which these flavors have replaced the traditional '*Tian Op* ' smoking or direct smoking methods. However, the variety of smoked desserts now available to the consumer is evidence of their increased usage in recent years.

The possible advantages of aqueous flavors over conventional or direct smoking methods have been considered by Draudt (1963) and Hollenbeck (1963). According to the latter author, the major benefit to be derived is flavor reproducibility, which can be rigidity controlled. At least one of the manufacturing processes used in the preparation of the aqueous flavors has been described in detail in the literature (Hollenbeck, 1963). It consists of burning wood with a limited amount of air to form smoke. The chemical composition of these latter products has not been established; however, the major components are believed to be condensates of phenols and carbonyl compounds (Hollenbeck, 1963).

The demonstrated presence of the carcinogen, benzo[a]pyrene, and of other polycyclic hydrocarbons in smoked food has stimulated interest in the analysis of liquid smoke flavors. Lijinsky and Shubik (1965) isolated various hydrocarbons including pyrene, fluoranthene, benzo perylene, chrysene and benzo[a]pyrene, as well as many of the aforementioned hydrocarbons. Analyses of various samples of smoked fish and ham showed the presence of trace quantities of benzo[a]pyrene as well as other hydrocarbon types (Lijinsky and Shubik, 1965).

Propylene glycol, also known by the systematic name of propane-1,2-diol, has two hydrophilic alcoholic hydroxyl groups (OH^-). It is an organic compound (a diol alcohol), and is usually a tasteless, odorless, and colorless, clear, oily liquid that is hygroscopic and miscible with water, acetone and chloroform; it is manufactured by the hydration of propylene oxide. Propylene glycol is generally recognized as safe by the United States Food and Drug Administration for use as a direct food additive under the conditions prescribed and was also approved for certain indirect food additive uses (U.S. Food and Drug Administration, 2012). Propylene glycol has a wide range of practical applications in: antifreeze, coolants and aircraft de-icing fluids; heat transfer and hydraulic fluids; solvents; food; flavors and fragrances; cosmetics and personal care products; pharmaceuticals; chemical intermediates; plasticizers; and thermoset plastic formulations (Greenwood and Earnshaw, 1997). Dainius *et al.* (2006) developed a method of producing a liquid smoke from wood tar for use in food processing using propylene glycol as a trapping solvent. The liquid smoke product that was produced contained neither detectable amounts of 3,4-benzopyrene nor of those components of the heavy, essentially water-insoluble material that settled out of an aqueous condensation of wood smoke, most of which were soluble in propylene glycol.

The dessert is smoked in a closed container for 30–60 min and about 3–5 times depending on the type and quantity of the dessert. The smoking time reduces the shelf life of the dessert because bacteria can grow during this process. Other disadvantages are the intensity of flavor is not the same in every piece of dessert and the carbon from the candle used in the smoking process can stick to the dessert surface. With liquid smoke, the intensity of the aroma in a product can be controlled, which contributes to process control in the food industry. The benefits of liquid smoke are reduced smoking time, increased ease of utilization and standardization of the intensity of the flavor in a product.

In order to assure the safety of liquid smoke use, the current study involving a subchronic toxicity test using laboratory animals was undertaken by the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand. If not toxic, this product will be beneficial to the Thai dessert industry.

MATERIALS AND METHODS

Chemicals

An amount of 99.5% pure propylene glycol (PG) was obtained from Italmar Co., Ltd., Thailand. Beeswax was obtained from the Sayun Bee Farm (Chiang Mai, Thailand), with composition and properties reported by the supplier being: yellow color, a saponification value of 87–110 mg KOH.g^{-1}, an acid value of 17–24 mg KOH.g^{-1}, an ester value of 70–86 mg KOH.kg^{-1}, a melting point of 62–65 °C and impurities of 0.1%. Kaffir lime fruits were purchased from a local market (Pathum Thani, Thailand) and the peel was immediately removed and dried for 36 hr at 35 °C in a tray dryer to a moisture content of 12%. The dried peel was milled, sieved through a 0.25 mesh sieve and stored in a desiccator until needed. Benzoin was purchased from a local market in Bangkok, Thailand (originating from Chiang Kwang, Laos). Sandalwood, originating from the Chumpon province in Thailand, was obtained from the Thai Public Health Ministry (Bangkok, Thailand), then milled, sieved through a 0.25 mesh and stored in a desiccator until needed.

Preparation of liquid smoke

A '*Tian Op* ' candle and liquid smoke were prepared according to a method described by Watcharananun and Haungrak (2009). The smoke was generated from manual lighting of the candle for 30 s and then it was drawn by vacuum (pump) at the rate of 50 mL.min^{-1} into a 100 mL flask containing propylene glycol (30 g). The liquid smoke was diluted with PG and adjusted to the desired concentrations for the subchronic toxicity test.

Experimental animals

For the test, 120 Wistar rats (60 male and 60 female) weighing 180 ± 20 g each were purchased from the National Laboratory Animal Center, Mahidol University, Salaya, Thailand, The animals were housed at the Laboratory Animal Center, National Institute of Health, Department of Medical Sciences at 25 ± 1 ºC and 60% humidity under a 12 hr-light-dark cycle. They were given commercial pellet diets and water was given freely. Prior to the experiment, they were randomly divided into five groups each containing 12 animals of each sex.

Subchronic toxicity study

A 90-day subchronic toxicity study on liquid smoke (LS) was conducted according to the World Health Organization guidelines for toxicity investigation of herbal medicines (World Health Organization, 2000). Five groups of rats were used. Groups 1 and 2 were water and PG control groups intragastrically receiving distilled water and PG at a volume of 10 mL.kg^{-1}.day^{-1}, respectively, where the weight was based on live body weight. Groups 3–5 were experimental groups intragastrically administered with LS at doses of 0.04, 0.4 and 4.0 mL.kg^{-1}.day^{-1} which were equivalent to 1, 10, and 100 fold usage by humans (calculation based on 50 kg body weight), respectively for 90 consecutive days. During the period of the experiment, body weight and food consumption were recorded weekly and the rats were closely observed for signs of abnormalities. At the end of the 90-day treatment period, the animals were fasted for 18 hr and then anaesthetized with ethyl ether and blood samples collected from post vena cava for analyzing hematological and serum clinical chemistry values. These were carried out using an automatic hematological analyzer (Cell Dyn® 3500; GMI Inc., Ramsey, MN, USA.) and an automatic chemistry analyzer (Hitachi® 912; GMI Inc., Ramsey, MN, USA.) respectively. Hematological parameters determined were %hematocrit, hemoglobin, red blood cells (RBC), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), total leukocyte (WBC), %neutrophil, %eosinophil, lymphocyte, %monocyte, %basophil and platelet counts. Clinical chemistry values measured were alkaline

phosphatase (ALP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), total protein, albumin, globulin, total bilirubin, blood urea nitrogen (BUN), creatinine, glucose, uric acid, triglycerides, total cholesterol, sodium, potassium and chloride.

Necropsy was performed to examine gross pathological lesions of various internal organs (brain, heart, lung, liver, stomach, spleen, kidneys spleen, bladder, ovary and uterus in the female rats, testis in the male) and adrenal glands were weighed and determined in terms of relative organ weight (g/kg body weight). As well as the organs mentioned above, trachea, esophagus, pancreas, intestine, seminal vesicles, thyroid gland, lacrimal, salivary and mammary glands were preserved in 10% buffered formalin solution and were subsequently sent for histological preparation for tissue slides. The tissue slides were histopathologically examined by a veterinarian pathologist at the Medicinal Plant Research Institute, Department of Medical Science, Ministry of Public Health, Thailand.

Statistical analysis

All data were expressed as mean ± SD. The values obtained from different groups were statistically analyzed using one-way analysis of variance and multiple comparisons with a Bonferroni test at $P < 0.05$. Histopathological results were expressed as the number of rats with pathological findings per total number of rats treated (incidences) and were statistically analyzed using Fishers Exact Test at $P < 0.05$.

RESULTS

Effect of liquid smoke on hematological values

Wistar rats receiving LS at all dosages had no significant differences between average body weight when compared with the water control and PG-treated rats. Rats receiving LS at any dosage had significantly less food consumption than the water control group in most of the experimental periods and so did rats receiving PG. However, the food consumption of rats receiving LS was not significantly less when compared with PG-treated rats. Both male and female rats receiving LS showed no abnormal behavior or clinical signs throughout the period of experiment.

Both male and female rats receiving LS at any dosage had no significant differences between hematological parameters when compared with their water control and PG-treated groups except for the male rats receiving 0.4 $mL.kg^{-1}$ LS which had significantly higher %monocyte than in the water control group. Hematological values are summarized in Tables 1 and 2.

Effect of liquid smoke on clinical chemistry values

Male rats receiving LS at doses of 0.04 and 0.4 $mL.kg^{-1}.day^{-1}$ had significantly higher BUN when compared with their water control groups as did PG-treated rats. Creatinine levels were significantly higher in all LS-treated male rats and in PG-treated male rats when compared with their water control group. Albumin levels were significantly increased in male and female rats receiving LS at a dose of 0.4 and 4.0 $mL.kg^{-1}.day^{-1}$ and total protein was significantly increased in male rats receiving LS at a dose of 0.4 $mL.kg^{-1}.day^{-1}$ when compared with their water control group. Male rats receiving LS at a dose of 0.4 $mL.kg^{-1}.day^{-1}$ had significantly increased glucose level when compared with their water control group and also when compared with the PG-treated rats. Clinical chemistry values are summarized in Tables 3 and 4.

Effects of liquid smoke on visceral organs

At necropsy, no remarkable gross pathological lesions were found in LS-treated, PG-treated rats and water control groups. Male rats receiving LS at each dosage had significantly increased relative liver weight when compared with their water control group and so had the PG-

Table 1 Hematological values of male Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Parameter	Water	PG	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day)		
			0.04	0.4	4.0
	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 12)
Hematocrit (%)	52.68 ± 2.19	50.78 ± 2.59	50.87 ± 2.75	51.83 ± 3.42	53.45 ± 5.69
Hemoglobin (g.dL^{-1})	16.28 ± 0.54	15.87 ± 0.70	15.93 ± 0.74	16.14 ± 0.87	16.58 ± 1.40
RBC (×10^6 µL^{-1})	9.55 ± 0.43	9.44 ± 0.56	9.05 ± 0.40	9.54 ± 0.77	9.68 ± 1.14
MCV (µm^3/red cell)	55.14 ± 1.92	53.90 ± 1.98	56.23 ± 2.87	54.44 ± 1.92	55.33 ± 2.22
MCH (pg/red cell)	17.04 ± 0.64	16.83 ± 0.54	17.62 ± 0.80	16.96 ± 0.63	17.21 ± 0.90
MCHC (g/dL RBC)	30.91 ± 0.41	31.25 ± 0.50	31.35 ± 0.35	31.16 ± 0.49	31.08 ± 0.73
WBC (cells.µL^{-1})	3.41 ± 1.02	3.37 ± 0.82	3.23 ± 0.71	3.28 ± 0.55	3.05 ± 0.67
Neutrophil (%)	19.39 ± 5.01	20.44 ± 4.57	22.80 ± 6.50	21.33 ± 3.06	23.08 ± 4.75
Eosinophil (%)	1.51 ± 0.53	1.39 ± 0.68	1.70 ± 0.68	1.75 ± 0.91	1.32 ± 0.47
Lymphocyte (%)	75.72 ± 6.79	73.89 ± 6.42	71.04 ± 8.16	68.55 ± 4.43	70.58 ± 6.56
Monocyte (%)	2.40 ± 2.37	2.93 ± 2.37	3.27 ± 2.44	6.27 ± 3.74*	3.59 ± 3.06
Basophil (%)	0.97 ± 0.50	1.35 ± 0.93	1.20 ± 0.69	2.10 ± 1.24	1.42 ± 1.22
Platelet (cells.µL^{-1})	1039.58 ± 82.32	997.29 ± 164.41	923.54 ± 124.41	1098.88 ± 164.93	1023.82 ± 183.78

Values are expressed as mean ± SD.

RBC = Red blood cells; MCV = mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; WBC = Total leukocyte; PG = Propylene glycol; BW = Body weight.

* = Significant difference from water control group ($P < 0.05$).

Table 2 Hematological values of female Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Parameter	Water (mL per kg BW per day)	PG (mL per kg BW per day)	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day)		
	10	10	0.04	0.4	4.0
	(n = 12)	(n = 12)	(n = 12)	(n = 12)	(n = 11)
Hematocrit (%)	51.22 ± 2.65	49.28 ± 4.08	49.34 ± 1.83	50.99 ± 2.90	49.99 ± 3.64
Hemoglobin (g/dL)	15.95 ± 0.76	15.40 ± 1.07	15.45 ± 0.55	15.91 ± 0.84	15.58 ± 1.11
RBC (×10^6 µL^{-1})	8.80 ± 0.47	8.48 ± 0.80	8.57 ± 0.47	8.64 ± 0.44	8.61 ± 0.48
MCV (µm^3/red cell)	58.23 ± 1.02	58.18 ± 1.52	57.64 ± 1.91	59.02 ± 1.62	58.04 ± 1.27
MCH (pg/red cell)	18.16 ± 0.43	18.20 ± 0.60	18.10 ± 0.67	18.43 ± 0.57	18.09 ± 0.47
MCHC (g/dL RBC)	31.18 ± 0.42	31.30 ± 0.49	31.42 ± 0.19	31.22 ± 0.45	31.20 ± 0.53
WBC (cells.µL^{-1})	2.51 ± 0.59	2.28 ± 0.51	2.05 ± 0.54	2.29 ± 0.70	2.05 ± 0.51
Neutrophil (%)	22.21 ± 7.81	25.74 ± 6.96	26.10 ± 5.74	24.93 ± 7.15	22.75 ± 5.16
Eosinophil (%)	1.07 ± 0.52	1.34 ± 0.57	1.21 ± 0.48	0.97 ± 0.32	1.20 ± 0.52
Lymphocyte (%)	70.94 ± 7.75	69.09 ± 6.99	67.61 ± 6.99	70.55 ± 6.03	72.15 ± 6.09
Monocyte (%)	4.23 ± 3.09	2.65 ± 1.86	3.39 ± 2.36	2.46 ± 3.04	2.67 ± 1.61
Basophil (%)	1.55 ± 1.34	1.18 ± 0.64	1.76 ± 1.07	1.10 ± 1.01	1.28 ± 0.66
Platelet (cells.µL^{-1})	951.71 ± 123.67	977.13 ± 131.28	940.38 ± 121.22	914.13 ± 102.97	933.54 ± 72.47

Values are expressed as mean ± SD.

RBC = Red blood cells; MCV = mean corpuscular volume; MCH = Mean corpuscular hemoglobin; MCHC = Mean corpuscular hemoglobin concentration; WBC = Total leukocyte; PG = Propylene glycol; BW = Body weight.

* = Significant difference from water control group ($P < 0.05$).

Table 3 Clinical chemistry values of male Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Parameter	Water (mL per kg BW per day) 10 (n = 12)	PG (mL per kg BW per day) 10 (n = 12)	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day) 0.04 (n = 12)	0.4 (n = 12)	4.0 (n = 12)
ALT($U.L^{-1}$)	32.67 ± 3.82	37.67 ± 8.17	33.33 ± 4.60	36.25 ± 2.77	32.91 ± 7.16
AST($U.L^{-1}$)	91.42 ± 14.56	91.67 ± 15.68	87.42 ± 10.96	91.42 ± 9.50	89.27 ± 12.27
ALP($U.L^{-1}$)	58.58 ± 7.74	65.92 ± 9.93	60.67 ± 11.11	65.25 ± 7.00	69.09 ± 12.41
BUN ($mg.dL^{-1}$)	17.52 ± 1.69	21.71 ± 3.46*	22.77 ± 3.73*	21.86 ± 3.20*	21.32 ± 4.61
Creatinine (mg %)	0.58 ± 0.05	0.72 ± 0.09*	0.71 ± 0.13*	0.71 ± 0.11*	0.71 ± 0.11*
Albumin ($g.dL^{-1}$)	4.46 ± 0.13	4.61 ± 0.16	4.63 ± 0.15	4.75 ± 0.17*	4.68 ± 0.05*
Bilirubin ($mg.dL^{-1}$)	0.11 ± 0.03	0.10 ± 0.03	0.11 ± 0.04	0.12 ± 0.04	0.13 ± 0.05
Total protein (g/dl)	6.88 ± 0.21	7.17 ± 0.25	7.12 ± 0.35	7.23 ± 0.35*	7.10 ± 0.28
Glucose ($mg.dL^{-1}$)	221.84 ± 47.08	329.93 ± 102.02*	316.62 ± 114.01	405.11 ± 79.97*	324.04 ± 101.67
Uricacid ($mg.dL^{-1}$)	4.82 ± 1.10	4.31 ± 1.99	4.15 ± 2.09	5.78 ± 1.65	4.57 ± 1.34
Triglyceride($mg.dL^{-1}$)	82.70 ± 31.14	103.45 ± 45.43	93.17 ± 33.63	140.07 ± 88.06	92.25 ± 37.57
Cholesterol ($mg.dL^{-1}$)	67.52 ± 15.43	71.13 ± 11.84	74.13 ± 8.25	76.22 ± 18.35	76.85 ± 15.15
Na^{+}($mmol.L^{-1}$)	147.08 ± 2.19	145.58 ± 2.23	145.92 ± 1.73	147.25 ± 2.05	148.09 ± 1.58
K^{+}($mmol.L^{-1}$)	6.84 ± 0.66	6.25 ± 1.37	6.40 ± 0.90	6.42 ± 1.00	6.69 ± 1.39
Cl^{-}($mmol.L^{-1}$)	103.67 ± 2.61	101.00 ± 1.91*	101.33 ± 1.23	101.17 ± 2.29	102.18 ± 2.36

Values are expressed as mean ± SD.

PG = Propylene glycol; BW = Body weight; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; BUN = Blood urea nitrogen.

* = Significant difference from water control group ($P < 0.05$).

Table 4 Clinical chemistry values of female Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 days.

Parameter	Water (mL per kg BW per day) 10 (n = 12)	PG (mL per kg BW per day) 10 (n = 12)	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day) 0.04 (n = 12)	0.4 (n = 12)	4.0 (n = 11)
ALP($U.L^{-1}$)	28.25 ± 9.82	25.83 ± 4.22	29.42 ± 7.87	24.92 ± 6.50	27.75 ± 5.64
ALT($U.L^{-1}$)	31.42 ± 7.98	27.08 ± 3.37	26.75 ± 3.19	30.67 ± 12.30	27.67 ± 5.09
AST($U.L^{-1}$)	97.33 ± 16.56	87.92 ± 14.43	83.00 ± 9.94	92.42 ± 35.05	85.17 ± 12.55
BUN ($mg.dL^{-1}$)	23.15 ± 5.27	25.33 ± 6.58	25.71 ± 4.42	30.00 ± 7.68	29.12 ± 6.43
Creatinine (mg %)	0.65 ± 0.10	0.67 ± 0.16	0.68 ± 0.09	0.74 ± 0.16	0.68 ± 0.11
Total protein ($g.dL^{-1}$)	6.90 ± 0.29	6.97 ± 0.31	7.01 ± 0.31	7.25 ± 0.28	7.18 ± 0.34
Albumin ($g.dL^{-1}$)	4.86 ± 0.23	5.07 ± 0.27	5.11 ± 0.26	5.28 ± 0.24*	5.26 ± 0.25*
Bilirubin ($mg.dL^{-1}$)	0.13 ± 0.06	0.15 ± 0.07	0.13 ± 0.04	0.14 ± 0.04	0.16 ± 0.07
Glucose ($mg.dL^{-1}$)	124.68 ± 35.96	149.15 ± 25.63	132.97 ± 26.04	138.75 ± 32.45	137.86 ± 29.78
Uric acid ($mg.dL^{-1}$)	3.59 ± 0.87	2.73 ± 1.21	2.58 ± 0.99	3.10 ± 1.07	2.88 ± 1.17
Triglyceride($mg.dL^{-1}$)	43.51 ± 7.06	37.67 ± 9.89	34.70 ± 6.84	38.72 ± 12.57	56.07 ± 11.78
Cholesterol($mg.dL^{-1}$)	58.96 ± 12.13	62.77 ± 12.69	64.17 ± 12.06	68.64 ± 8.67	56.07 ± 11.78
Na^{+}($mmol.L^{-1}$)	146.92 ± 2.28	145.33 ± 1.16	146.83 ± 1.90	146.17 ± 1.90	147.42 ± 1.24
K^{+}($mmol.L^{-1}$)	8.18 ± 1.69	6.71 ± 2.14	6.42 ± 1.38	7.28 ± 1.62	6.91 ± 2.11
Cl^{-}($mmol.L^{-1}$)	107.17 ± 1.70	105.25 ± 1.42	105.17 ± 1.80	105.33 ± 2.06	105.08 ± 2.35

Values are expressed as mean ± SD.

PG = Propylene glycol; BW = Body weight; ALT = Alanine aminotransferase; AST = Aspartate aminotransferase; ALP = Alkaline phosphatase; BUN = Blood urea nitrogen.

* = Significant difference from water control group ($P < 0.05$).

treated rats. Male rats receiving LS at a dose of 0.04 mL.kg^{-1}.day^{-1} had significantly more right kidney relative weight than their water control groups (Table 5). No alterations of relative organ weight were found in the female rats receiving LS (Table 6).

Effects of Liquid smoke on organs histopathology

Incidences of histopathological alterations in some organs of rats receiving any dosage of LS were not significantly different from those of their water control group and PG-treated rats. In addition, the histopathological changes observed in this study were not indicative of LS toxicity, that is, congestion in the heart, liver and kidney. Histopathological results are summarized in Tables 7 and 8.

DISCUSSION

The subchronic toxicity study indicated that LS did not affect the food consumption and average body weight of Wistar rats. The increase of monocyte only in male rats receiving 0.4 mL.kg^{-1} was not dose dependent; therefore this alteration could not contribute to LS. The significantly higher BUN in male rats receiving 0.04 and 0.4 mL.kg^{-1} LS and also significantly higher creatinine in male rats receiving all dosages of LS when compared with their water control group could not be attributable to LS effects. Such alterations were not dosage dependent and did not differ from the PG-treated rats in a dosage-dependent manner. Male and female rats receiving LS at 0.4 and 4.0 mL.kg^{-1} had significantly higher albumin levels than their water control groups; however, their albumin levels were not significantly higher than for the PG-treated groups. In addition, such

Table 5 Body weight and relative organ weight of male Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Organ	Water (mL.kg BW^{-1}.day^{-1})	PG (mL.kg BW^{-1}.day^{-1})	Dose of liquid smoke of '*Tian Op* ' (mL.kg BW^{-1}.day^{-1})		
	10 (n = 12)	10 (n = 12)	0.04 (n = 12)	0.4 (n = 12)	4.0 (n = 12)
Initial body weight (g)	194.06 ± 6.28	192.67 ± 10.64	190.52 ± 8.01	193.95 ± 9.63	191.17 ± 9.64
Final body weight (g)	493.00 ± 34.10	506.06 ± 37.90	485.04 ± 43.15	517.16 ± 38.94	491.65 ± 44.68
Brain (g.kg^{-1})	4.23 ± 0.29	4.18 ± 0.33	4.36 ± 0.37	4.15 ± 0.32	4.27 ± 0.36
Heart (g.kg^{-1})	2.67 ± 0.16	2.68 ± 0.20	2.85 ± 0.26	2.70 ± 0.14	2.68 ± 0.39
Lung (g.kg^{-1})	3.43 ± 0.27	3.39 ± 0.23	3.52 ± 0.44	3.36 ± 0.26	3.55 ± 0.45
Liver (g.kg^{-1})	28.70 ± 1.89	33.56 ± 2.66*	34.49 ± 3.17*	35.14 ± 2.82*	35.08 ± 3.53*
Stomach (g.kg^{-1})	3.89 ± 0.36	3.93 ± 0.23	4.08 ± 0.24	4.08 ± 0.73	4.08 ± 0.40
Spleen (g.kg^{-1})	1.72 ± 0.16	1.78 ± 0.98	1.90 ± 0.23	1.68 ± 0.16	1.82 ± 0.20
Right kidney (g.kg^{-1})	2.49 ± 0.19	2.56 ± 0.18	2.73 ± 0.14*	2.64 ± 0.17	2.70 ± 0.19
Left kidney (g.kg^{-1})	2.44 ± 0.16	2.51 ± 0.19	2.61 ± 0.12	2.51 ± 0.17	2.56 ± 0.17
Right testis (g.kg^{-1})	6.25 ± 0.55	5.75 ± 0.56	6.17 ± 0.45	5.89 ± 0.52	5.92 ± 0.64
Left testis (g.kg^{-1})	6.23 ± 0.67	5.74 ± 0.49	6.18 ± 0.47	5.74 ± 0.42	5.94 ± 0.78
Right adrenal gland (g.kg^{-1})	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Left adrenal gland (g.kg^{-1})	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01
Bladder (g)	0.31 ± 0.10	0.34 ± 0.06	0.28 ± 0.08	0.28 ± 0.04	0.29 ± 0.06

Values are expressed as mean ± SD.

PG = Propylene glycol; BW = Body weight.

* = Significantly different from water control group ($P < 0.05$).

alterations were not clearly dosage dependent and were not indicative of any abnormal liver function. The increased glucose levels in male rats receiving 0.4 mL.kg^{-1} were not dosage dependent and therefore, this phenomenon did not contribute to LS.

Although relative liver weights were significantly increased in all LS-treated male rats

Table 6 Body weight and relative organ weight of female Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Organ	Water (mL per kg BW .day^{-1}) 10 (n = 12)	PG (mL per kg BW .day^{-1}) 10 (n = 12)	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day) 0.04 (n = 12)	0.4 (n = 12)	4.0 (n = 11)
Initial body weight (g)	168.14 ± 7.56	167.33 ± 5.44	165.28 ± 5.90	166.15 ± 9.41	166.83 ± 4.97
Final body weight (g)	259.10 ± 16.47	264.55 ± 14.82	262.06 ± 12.18	262.36 ± 11.62	263.98 ± 13.78
Brain (g.kg^{-1})	7.24 ± 0.62	7.07 ± 0.47	7.14 ± 0.48	7.18 ± 0.44	7.10 ± 0.47
Heart (g.kg^{-1})	3.24 ± 0.16	3.17 ± 0.31	3.12 ± 0.14	3.39 ± 0.30	3.14 ± 0.14
Lung (g.kg^{-1})	4.62 ± 0.31	4.49 ± 0.51	4.41 ± 0.34	4.60 ± 0.44	4.59 ± 0.31
Liver (g.kg^{-1})	31.08 ± 3.03	31.96 ± 3.49	29.60 ± 3.44	32.52 ± 3.20	30.68 ± 3.69
Stomach (g.kg^{-1})	5.45 ± 0.36	5.52 ± 0.56	5.60 ± 0.56	5.69 ± 0.41	5.62 ± 0.45
Spleen (g.kg^{-1})	2.26 ± 0.21	2.24 ± 0.22	2.15 ± 0.29	2.22 ± 0.27	2.24 ± 0.26
Right kidney (g.kg^{-1})	3.01 ± 0.23	3.17 ± 0.23	3.19 ± 0.21	3.18 ± 0.17	3.15 ± 0.29
Left kidney (g.kg^{-1})	2.93 ± 0.16	3.06 ± 0.17	3.03 ± 0.14	3.05 ± 0.21	3.01 ± 0.28
Right adrenal gland (g.kg^{-1})	0.14 ± 0.02	0.14 ± 0.02	0.13 ± 0.03	0.13 ± 0.02	0.15 ± 0.02
Left adrenal gland (g.kg^{-1})	0.15 ± 0.02	0.15 ± 0.02	0.15 ± 0.03	0.14 ± 0.02	0.15 ± 0.02
Bladder (g.kg^{-1})	0.31 ± 0.04	0.28 ± 0.04	0.29 ± 0.05	0.31 ± 0.05	0.28 ± 0.03
Uterus (g.kg^{-1})	2.54 ± 0.52	2.11 ± 0.82	2.30 ± 0.90	2.36 ± 0.61	2.23 ± 0.84
Right ovary (g.kg^{-1})	0.30 ± 0.08	0.28 ± 0.05	0.28 ± 0.06	0.26 ± 0.05	0.25 ± 0.04
Left ovary (g.kg^{-1})	0.32 ± 0.12	0.27 ± 0.05	0.28 ± 0.05	0.26 ± 0.05	0.26 ± 0.03

Values are expressed as mean ± SD.

PG = Propylene glycol; BW = Body weight.

Table 7 Histopathological results of male Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Organ	Microscopic findings	Water (mL per kg BW per day) 10	PG (mL per kg BW per day) 10	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day) 0.04	0.4	4.0
Lung	BALT	5/12	2/12	6/12	7/12	6/12
Heart	Congestion	1/12	4/12	4/12	4/12	3/12
Liver	Congestion	9/12	4/12	10/12	9/12	8/12
Kidney	Congestion	11/12	12/12	12/12	12/12	10/12
Small intestine	GALT	2/12	2/12	1/12	0/12	2/12
Large intestine	GALT	2/12	2/12	1/12	0/12	2/12
Adrenal gland	Cortical fatty infiltration	4/12	7/12	6/11	2/12	2/12

Results are expressed as number of rats with pathological lesions per total number of rats

PG = Propylene glycol; BW = Body weight.

BALT = Bronchial-associated lymphoid tissue; GALT = Gut-associated lymphoid tissue.

when compared with those of their water control group, the increments were not dosage dependent. It was also found that the PG-treated rats had significantly more relative liver weight than the water control group. Taken together, this alteration may not contribute to LS. The histopathological results showed alterations in some organs (Tables 7 and 8). Since the incidences of microscopic findings in LS-treated rats did not show any statistical differences when compared with those of the water control and PG-treated groups, and were not dosage dependent with the increasing LS dosages, these histopathological changes were not suggestive of LS.

Propylene glycol was reported by Werley *et al.* (2011) to be safe in non-clinical trials with rats They studied safety and pharmacokinetic evaluations of propylene glycol aerosol and found that PG elimination in the high dose groups (a mass median aerodynamic diameter of 2.29 μm, with a 1.56 geometric standard deviation) in the rat studies showed terminal plasma and lung concentration-time profiles suggesting a zero-order elimination process. There was no apparent tissue toxicity of the lung, liver and kidney in these studies. Under the conditions of these studies, the no observed effect level for rats was determined to be 20 $mg.kg^{-1}.day^{-1}$ for the 28-day study and the chronic toxicity/oncogenicity studies did not identify any tumors (Spencer, 2005)

The safety of propylene glycol has also been studied in cows; Kristensen and Raun (2007) reported that four lactating Holstein cows fitted with ruminal cannulas and permanent indwelling catheters in the mesenteric artery, mesenteric vein, hepatic portal vein and hepatic vein, were used in a cross-over design to study the metabolism of propylene glycol (PG). Each cow received two treatments—namely, a control (no infusion) and infusion of 650 g of PG into the rumen at the time of the morning feeding. Propylene glycol was infused on the day of sampling only. Samples of arterial, portal and hepatic blood as well as ruminal fluid were obtained at 0.5 hr before feeding and at 0.5, 1.5, 2.5, 3.5, 5, 7, 9 and 11 hr after feeding. Infusion of PG did not affect the ruminal pH or the total concentration of ruminal volatile fatty acids, but did decrease the molar proportion of ruminal acetate. The ruminal concentrations of PG, propanol and propanal it can change to propinoateas well as the molar proportion of propionate increased with PG infusion. The plasma concentrations of PG, ethanol, propanol, propanal, glucose, L-lactate, propionate and insulin increased with PG and the plasma concentrations of acetate and β-hydroxybutyrate decreased. The net portal

Table 8 Histopathological results of female Wistra rats treated with liquid smoke of '*Tian Op* ' for 90 d.

Organ	Microscopic findings	Water (mL per kg BW per day)	PG (mL per kg BW per day)	Dose of liquid smoke of '*Tian Op* ' (mL per kg BW per day)		
		10	10	0.04	0.4	4.0
Lung	BALT proliferation	6/12	4/12	5/12	7/12	3/11
Heart	Congestion	4/12	1/12	0/12	2/12	0/11
Liver	Congestion	9/12	11/12	10/12	10/12	11/11
Kidney	Congestion	10/12	12/12	11/12	12/12	11/11
	Tubular casts	3/12	2/12	3/12	2/12	0/11
Small intestine	GALT proliferation	1/12	3/12	0/12	1/12	0/11
Large intestine	GALT proliferation	1/12	2/12	0/12	0/12	2/11
Adrenal gland	Cortical congestion	1/12	0/12	1/12	1/12	1/11

Results are expressed as number of rats with pathological lesions per total number of rats

PG = Propylene glycol; BW = Body weight.

BALT = Bronchial-associated lymphoid tissue; GALT = Gut-associated lymphoid tissue.

flux of PG, propanol and propanal increased with PG. The hepatic uptake of PG was equivalent to 19% of the intraruminal dose. When cows were dosed with PG, the hepatic extraction of PG was between 0 and 10% depending on the plasma concentration of PG, explaining the slow decrease in arterial PG. The increased net hepatic flux of L-lactate with PG could account for the entire hepatic uptake of PG, which suggests that the primary hepatic pathway for PG is oxidation to L-lactate. The hepatic uptake of propanol increased with PG, but no effects of PG on the net hepatic and net splanchnic flux of glucose were observed. Despite no effect of PG on the net portal flux and net hepatic flux of propionate, the net splanchnic flux of propionate increased and the data suggested that propionate produced from hepatic metabolism of propanol is partly released to the blood. The data suggest that PG affects a cow's metabolism by two modes of action: 1) increased supply of L-lactate and propionate to gluconeogenesis and 2) insulin resistance of peripheral tissues induced by increased concentrations of PG and propanol as well as a decreased ratio of ketogenic to glucogenic metabolites in arterial blood plasma.

Kristensen and Raun (2007) further reported that not only did propylene glycol (PG) not pose a safety hazard, but also it increased the metabolism, as PG was metabolized in the rumen and liver and it affected metabolites, hormones, liver composition, feed intake and milk production. They evaluated whether PG was likely to prevent excessive fat mobilization and imbalances in the carbohydrate and fat metabolism and thereby reduce the risk of ketosis. PG decreased the molar ratio of acetate to propionate in rumen volatile fatty acids because part of PG was metabolized to propionate in the rumen. The remaining PG was absorbed directly from the rumen without alteration and entered gluconeogenesis via pyruvate. Oral administration of PG increased insulin by 200–400% within 30 min after drenching, indicating that PG is absorbed rather quickly. Allocation of PG also increased plasma glucose, although the response was limited, probably because of the large increase in insulin. PG decreased plasma concentrations of non-esterified fatty acids (NEFA) and beta-hydroxybutyrate, especially in early lactating cows with relatively high levels of NEFA. PG also reduced the triacylglycerol content of the liver and the concentrations of ketone bodies in milk and hence, has anti-ketogenic properties. Generally, PG had no statistically significant effect on milk production, but for cows in early lactation, PG tended to increase the milk yield and reduce the milk fat percentage, while the milk protein percentage was unchanged. Thus, PG had no effect on the energy-corrected milk yield. In general, PG did not affect feed intake. Together with the anti-ketogenic properties of PG, this suggests that PG may reduce the risk of subclinical and clinical ketosis (Nielsen *et al.,* 2004).

It should be noted that liquid smoke produced with beeswax generated the greatest amount of carcinogenic polyclyclic aromatic hydrocarbons (PAHs) at 0.13 $\mu g.kg^{-1}$ (Watcharananun and Haungrak , 2009); however, this was considered a small amount (Guillén *et al.*, 2000). The only PAH with an acceptable limit of 10 $\mu g.kg^{-1}$, fixed by the Food and Agriculture Organization/World Health Organization, is benzo[a]pyrene, because it is highly carcinogenic (World Health Organization, 2000). This was also found to be present in poplar and beech liquid smoke; however, the concentrations were well below the acceptable limit (Guillén *et al.*, 2000).

CONCLUSION

Wistar rats receiving LS at all dosages had no significant differences in their average body weight when compared with the water control and PG treated rats. Wistar rats receiving LS at any dosage had no significant differences of hematological parameters when compared with their water control and PG-treated groups except male rats receiving 0.4 $mL.kg^{-1}$ LS had significantly ($P < 0.05$) higher %monocyte than

that of water control group. Clinical chemistry values were significantly ($P < 0.05$) higher in all LS-treated male rats and in PG-treated male rats when compared with those of their water control group. The histopathology of organs revealed no abnormalities related to liquid smoke toxicity.

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