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Sub-acute oral administration of *Cymbopogon citratus* stem infusion and its effects on blood biochemical parameters, body and organ weights in rats

(Pemberian oral sub-akut teh *Cymbopogon citratus* dan kesannya ke atas parameter biokimia darah, berat badan dan organ tikus)

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Keywords: *Cymbopogon citratus,* sub-acute toxicity, biochemical parameters, organ weight

Abstract

A study was conducted to determine the effects of lemon grass stem infusion (*Cymbopogon citratus*) in female *Sprague-Dawley* rats. The objective of the study was to develop a basic guideline for safe use of lemon grass stem infusion. A total of 18 female rats were fed with 13 and 130 mg/kg body weight (BW) of the infusion for 28 days. Control rats only received distilled water. Blood samples were collected to assess renal and liver functions. Repeated administration of lemon grass stem infusion to rats did not produce any significant change in their blood lipid profiles [cholesterol, high-density lipoprotein (HDL), low-density lipoprotein (LDL) and triglycerides], liver function parameters [aspartate transaminase (AST), alanine transaminase (ALT) and gamma-glutamyl transferase (GGT)], renal function parameters (creatinine, urea and uric acid), total protein and glucose concentrations. The intake of the infusion also did not show hepatotoxic and nephrotoxic signs on rats. The weight of rats receiving the dose at 130 mg/kg BW (which is equivalent to 10 times of normal human consumption), was almost similar to the control group. The rats survived the treatment without showing any ill-effect. Based on this study, lemon grass stem infusion was not toxic even at 10 times higher than normal human consumption.

Introduction

Malaysia is a country that has diverse flora that can be used to treat diseases. Herbs are gaining popularity worldwide as remedies for human diseases. They are believed to be beneficial and can cure diseases naturally. Many scientific studies are based on ethnomedical as indicated by the number of rigorous studies published. Likewise, about three quarters of the world population also rely mainly on herbs for their health care even though there are more potent medicines available for curing diseases (Philipson 1993).

Cymbopogon citratus, popularly called lemon grass, is a widely cultivated tropical perennial shrub, which originates in Malaysia, Indochina and

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Sri Lanka. The stems of lemon grass are commonly used as flavouring agent and condiment in Malaysian cuisine. In India, China, Guatemala and Brazil, the plant is commercially planted for use in aromatherapy, perfumery, and for extraction of essential oil. The main compound in lemon grass is citral, which constitutes 65–85%. Citral is believed to possess the most beneficial effect of lemon grass, i.e. as anticancer agent and an antioxidant. One study reported that water extract of *C. citratus* stem contained enough citral that can cause death to cancer cells (Sommer 2006). Apart from that, the plant extracts have also been used as agents for anti pyretic, anti helminthic, anti bacterial, anti nociceptive, anti constipation aids and anti malaria (Onabanjo et al. 1993).

In Nigeria, lemon grass is used to treat fever, jaundice, hypertension, diabetes mellitus and obesity (Viana et al. 2000; Adeneye and Agbaje 2007). Despite its traditional uses for treating diseases, the toxicity of the plant has not been reported. So far only studies on the toxicity of the leaves have been reported (Leite et al. 1986; Lagarto Parra et al. 2001; Adeneye and Agbaje 2007; Fandohan et al. 2008; Blanco et al. 2009). Evaluation of the toxic effects of plant extracts is indispensable in order to consider a treatment is safe, and to enable the definition of the effects of an acute overdose (Lagarto Parra et al. 2001). Besides, the use of herbs in the treatment of human diseases usually lacks supporting data from scientific research. Thus, this study was undertaken to determine the sub-acute toxicity effect of lemon grass stem infusion in rats.

Materials and methods

Preparation of lemon grass stem infusion

Fresh *Cymbopogon citratus* stems measuring about 12 cm, were collected from a farm in Beranang, Selangor. They were washed thoroughly under running tap water, sliced and oven-dried until 7–10% moisture content and ground into powder with a blender. The powder was then kept at –20 °C until further use.

The lemon grass stem infusion used in the experiment was freshly prepared twice a week. The powder was steeped in boiling water (100 °C) at two different doses, 13 mg/kg for the low dose and 130 mg/kg for the high dose. The infusion was left to stand for 10 min before being filtered. The dosage for the present study was formulated based on the normal human consumption of lemon grass tea in Brazil, i.e.13 mg/kg body weight (BW) (reference, year). Hence, lemon grass teas at 13 mg/kg and 130 mg/kg body weight were selected for the study (Formigoni et al. 1986). The infusion was allowed to cool to ambient temperature before feeding the rats.

Test animals

A total of 18 female *Sprague-Dawley* rats were purchased from the animal breeding laboratory of the Institute of Medical Research, Kuala Lumpur, Malaysia. The animals were left for a week for acclimatisation to the room conditions (temperature 21 ± 2 ºC and 12/12 h light/dark cycle) and fed with a standard pellet diet with water offered *ad libitum*. The animals were randomly assigned to the control and two treatment groups (6 rats/group) and housed in stainless steel wire-bottomed cages containing wood shavings for bedding. The use and care of the laboratory animals was conducted in accordance with the internationally accepted principles as stated in the WHO guidelines (WHO 2003).

Sub-acute toxicity test

The sub-acute toxicity test was conducted in accordance with the guidelines published by the Organization for Economic Cooperation and Development (OECD) No. 407, Issue Date 7/27/95 (Derelanko and Hollinger 2002). Control rats were given distilled water and the two treatment groups were given the lemon grass stem infusion at the low dose (13 mg/kg BW) and high dose (130 mg/kg BW) respectively. At the onset

of dosing, the rats weighed 208 ± 16 g each. The test was carried out for 28 days.

After 28 days of the feeding trial, the rats were fasted overnight (for at least 15–16 h), then anesthetized with ethyl ether. Their blood samples were collected, approximately 2 ml, from the posterior vena cava and they were then sacrificed for organ samples. Gross examination on the external surfaces of all visceral organs, thoracic, abdominal and pelvic cavities were carried out.

Body weight

The weight of each rat was recorded on day 0 and at weekly intervals throughout the course of the study. Food and water intakes were measured twice a week.

Organ relative weight

Following blood collection, the rats were sacrificed. Liver, kidney, heart and ovary (including uterus) in the female rats were removed. The macroscopic appearance of the organs was noted and their weights were recorded. Each organ's relative weight was obtained by dividing the final weight of organ with the final body weight of the rat.

Biochemical analyses

The blood samples were centrifuged at 4 °C (3,000 rpm, 5 min) to obtain the serum fraction which was analysed for blood chemistry parameters namely, urea, uric acid, glucose, creatinine, total protein, albumin, total bilirubin, cholesterol, triglycerides, alanine aminotransaminase (ALT), aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), low-density lipoprotein (LDL) and high-density lipoprotein (HDL). These parameters were determined using a blood chemistry analyser (Selectra E, Vitalab).

Statistical analysis

The experiment was arranged in a completely randomised design with six rats per treatment. Analysis of variance was conducted on the data collected and significant differences between the control and treatment means were determined using LSD (SAS version 9.1). A probability level of $p < 0.05$ was considered as significant.

Results and discussion

During the study, no deaths of treated rats were observed. Lemon grass stem infusion did not show any abnormal behaviour as there were no signs of behavioural changes observed in all treated rats. Studies by Carlini et al. (1986) and Leite et al. (1986) found that lemon grass leaves at doses up to 40 times higher than corresponding dosage taken by humans did not induce any ill effect. An absence of toxic effects was also noted in the offspring when the infusion was administered prior to mating or during pregnancy.

Body weight

All rats subjected to different doses of lemon grass stem infusion showed weight gain over time. Initial rat weight at the onset of dosing was 208 ± 16 g and increased to 260 ± 4 g with weight gain of 12 ± 2 g. Weight for treated and non-treated rat at particular age did not show any significant change (*Figure 1*). Increment in body weight is important as it determines the health status of the animal groups (Heywood 1983). Therefore, the results indicated a positive health status for both treated and control rats. Food and water consumption levels were similar in both control and treated animals. Results were consistent with previous studies on oral toxicity of lemon grass leaf extracts by Leita et al. (1986), Lagarto Parra et al. (2001), Adeneye and Agbaje (2007), Fandohan et al. (2008) and Blanco et al. (2009). At present, there has been no report on oral toxicity of lemon grass stem extract.

Organ relative weight

Comparison of organ weights between the treated and untreated groups of animals has been used to predict the toxic effect of the test material (Pfeifer and Neumann 1986). Any change in organ weight is an indicator

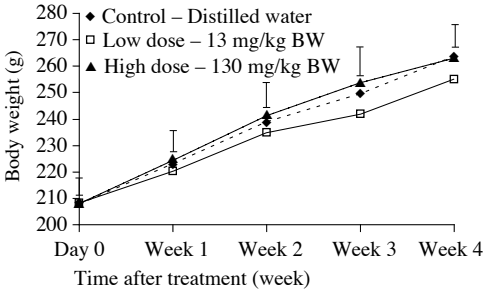

Figure 1. Changes in female Sprague-Dawley rats body weight over the duration of the treatments. Each point represents the mean of rats body weight with LSD value bar at p ≤0.05. No significance changes of rat body weight observed throughout the study

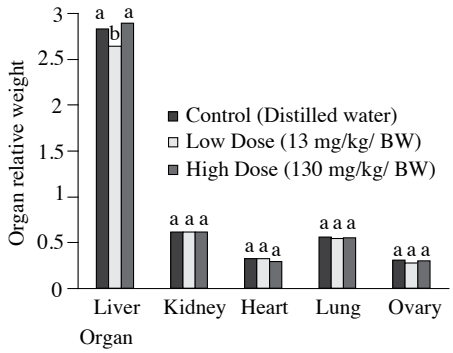

Figure 2. Organ relative weights at termination of the treatments. Each point represents the means ± standard deviation (SD)

of toxicity because organ weight will be affected by the suppression of body weight (Heywood 1983; Lu 1996). Data on relative organ weight measurements are presented in *Figure 2*. There were no remarkable differences in relative weights of liver, kidney, heart, lung and ovary. This indicated that organ relative weights were not affected by administration of lemon grass stem infusion.

Clinical pathology

No noticeable pathological changes such as organ swelling, atrophy or hypertrophy were observed by the naked eye on the main visceral organs in all treated rats during the study.

Blood biochemical analyses

Lipid profiles The blood lipid profiles determine whether the concentration of specific lipids present is abnormally high or low. Low level of cholesterol may associate with liver failure while high blood cholesterol may result from conditions such as biliary obstruction, diabetes mellitus and hypothyroidism. Low levels of triglycerides may indicate malabsorption or malnutrition while high level of triglycerides can be associated with diabetes mellitus and hypothyroidism (Jane and Mark 2011). Besides, screening of these two parameters also determines the risk of coronary heart disease caused by blockages of the blood vessels or harderning of the arteries (atherosclerosis) (AACC 2010). In the present study, there were no significant differences in blood lipid profiles between the control and treated rats (*Table 1*). This indicated that lemon grass stem infusion did not affect the blood lipid profiles of rats.

Liver function parameters Liver performs different kinds of biochemical, synthetic and excretory functions, so no single biochemical test can detect the overall functions of the liver. Liver function test is a non-invasive yet sensitive screening for liver dysfunction. Serum enzymes including AST and ALT are mainly used to detect injury to liver cells (hepatocytes). Under normal circumstances, these enzymes reside in the hepatocytes. However, these enzymes will spill into the blood stream if the liver is injured, thus raising their levels in the blood (Thapa and Walia 2007). Another enzyme is GGT, which acts as an indicator for cholestasis (e.g. biliary duct obstruction). Obstructed bile duct will induce the synthesis of GGT, thus elevateing the levels in blood (GPAC 2004). This study showed that the AST, ALT and GGT levels in rats treated with lemon grass stem infusion were not significantly different ($p > 0.05$) from those of the control rats (*Table 2*).

Bilirubin content was also evaluated to observe liver's capacity to transport

Table 1. Blood lipid profiles of female *Sprague-Dawley* rats at termination of the treatments

Dose (mg/kg)	HDL (mmol/litre)	Triglycerides (mmol/litre)	Cholesterol (mmol/litre)	LDL (mmol/litre)
Control	0.59a	0.55a	1.44a	0.60a
13	0.53a	0.48a	1.31a	0.56a
130	0.63a	0.42a	1.45a	0.64a

Mean values with the same letter within each column are not significantly different ($p \leq 0.05$) according to LSD

Table 2. Liver function parameters at termination of the treatments

Parameters	Dose (mg/kg)		
	Control	13	130
Total protein (g/litre)	57.76ab	53.87b	59.74a
Glucose (mmol/litre)	5.78a	5.90a	5.92a
Albumin (g/litre)	33.92a	31.75a	35.53a
Total bilirubin (μmol/litre)	2.13a	2.41a	2.41a
Globulin (g/litre)	24.00a	22.17a	24.00a
LDH (U/litre)	453.67a	378.00a	302.17a
AST (U/litre)	183.00a	177.67a	150.83a
ALT (U/litre)	60.17a	52.67a	59.83a
GGT (U/litre)	6.33a	7.17a	6.33a

Mean values with the same letter within each row are not significantly different ($p \leq 0.05$) according to LSD

Table 3. Renal function parameters at termination of the treatments

Dose (mg/kg)	Creatinine (mmol/litre)	Urea (mmol/litre)	Uric Acid (mmol/litre)
Control	74.70a	6.76a	0.13a
13	68.12a	6.48a	0.08a
130	77.02a	7.36a	0.13a

Mean values with the same letter within each column are not significantly different ($p \leq 0.05$) according to LSD

organic anions. Bilirubin is an endogenous anion derived from hemoglobin degradation from the red blood cells. Elevation of total bilirubin in blood showed impairment to the liver function (Thapa and Walia 2007) and this can also happen in cholestasis and hepatocellular injury (GPAC 2004). In this study, there was a slight elevation of total bilirubin in the treated female rats compared to the untreated group (*Table 2*). However this slight elevation was not significant ($p > 0.05$) indicating that there was no deterioration of liver function because the levels of ALT, AST and GGT were normal with no possibility of hepatic damage or biliary duct obstruction.

Liver biosynthetic capacity was also observed by the total protein content in the blood. In general, protein consists of albumin and globulin. The former is the most important protein in blood plasma synthesized by the liver and is a useful indicator of hepatic function (Howard 2010). A depressed albumin level maybe affected by the liver synthesis rate, degradation and volume of distribution (Thapa and Walia 2007). This pattern was not seen in the present study (*Table 2*), thus proving

that lemon grass stem infusion has no detrimental effect on the liver function of rats.

Renal function parameters The kidney is the second organ most affected by the actions of any compounds and chemicals. It performs many vital functions including removing metabolic wastes from the bloodstream, regulating the body's water balance and maintaining the pH of the body's fluid. These functions can be assessed by measuring the levels of plasma creatinine, urea and uric acid concentrations. Increments in these parameters indicate a diminishing ability of the kidneys to filter waste products from the blood and excrete them in the urine (Paula and Mark 2011). The present study showed that there were no significant differences in all three parameters for all groups of rats (*Table 3*).

Conclusion

Water extracts of lemon grass stem infusion showed no noticeable gross toxicity in the sub-acute oral test even at a dose 10 times higher than in normal human consumption. There was no significant difference in the blood biochemistry parameters for liver and kidney functions between the lemon grass-treated and control rats. Therefore, it can be concluded that the risk of toxicity from consuming lemon grass stem infusion is negligible.

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References

AACC (2010). Lipid profile. American Association for Clinical Chemistry. www. labtestonline. org. Retrieved on 2 March 2011 from http://??.

Adeneye, A.A. and Agbaje, E.O. (2007). Hypoglycemic and hypolipidemic effects of fresh leaf aqueous extract of *Cymbopogon citratus Stapf*. in rats. *Journal of Ethnopharmacology* 112: 440–444

Blanco, M.M., Costa, C.A.R.A., Freire, A.O., Santos Jr., J.G. and Costa, M. (2009). Neurobehavioral effect of essential oil of *Cymbopogon citratus* in mice. *Phytomedicine* 16: 265–270

Carlini, E.A., Contar Juida De, D.P., Silva-Filho, A.R., Da Silveira-Filho, N.G., Frochtengarten, M.L. and Bueno, O.F.A (1986). Pharmacology of lemongrass (*Cymbopogon citratus Stapf*) I. Effects of teas prepared from the leaves on laboratory animals. *Journal of Ethnopharmacology* 17: 37–64

Derelanko, M.J. and Hollinger, M.A. (2002). *Handbook of toxicology*. 2nd. Edition. Boca Raton New York: CRC Press

Fandohan, P., Gnonlonfin, B., Laleye, A., Gbenou, J.D., Darboux, R. and Moudachirau, M. (2008). Toxicity and gastric tolerance of essential oils from *Cymbopogon citratus*, *Ocimum gratissimum* and *Ocimum basilicum* in Wistar rats. *Food and Chemical Toxicology* 46: 2493–2497

Formigoni, M.L.O.S., Lodder, H.M., Filho, O.G., Ferreira, T.M.S. and Carlini, E.A. (1986). Pharmacology of lemongrass (*Cymbopogon citratus* Stapf). II. Effects of daily two month administration in male and female rats and in offspring exposed "in utero". *Journal of Ethnopharmacology* 17: 65–74

GPAC (2004). Liver chemistry and abnormalities in adults – evaluation and interpretation. Victoria: Guidelines and Protocols Advisory Committee

Heywood, R. (1983). Long term toxicity. In: *Animals and alternatives in toxicity testing* (Balls, M., Riddell, R.J. and Worden, A.N., eds.), p. 79–89. London: Academic Press

Howard, H. (2010). Liver function. Retrieved on 2 March 2011 from www.hrpca.org

Jane, E.P. and Mark, A.B. (2011). Lipid test. Retrieved on 2 March 2011 from www.surgeryencyclopedia.com

Leite, J.R., Seabra, V., De Lourdes, M., Maluf, E., Assolant, K.A., Sucheki, D., Klepacz, S., Tufik, S.K., Calil, H.M. and Carlini E.A. (1986). Pharmacology of lemongrass (*Cymbopogon citratus Stapf*.)

III. Assessment of eventual toxic, hypnotic and anxiolytic effects on humans. *Journal of Ethnopharmacology* 17: 75–83

Lagarto Parra, A., Silva Yhebra, R., Guerra Sardinas, I. and Iglesias Buela, L. (2001). Comparative study of the assay of *Artemia salina* L. and the estimate of the medium lethal dose (LD_{50} value) in mice, to determine oral acute toxicity of plant extracts. *Phytomedicine* 8(5): 395–400

Lu, F.C. (1996). *Basic toxicology – fundamentals, target organs and risk assessment*. Washington: Taylor and Francis

Onabanjo, A.O., Agbaje, E.O. and Odusote, O.O. (1993). Effects of aqueous extracts of *Cymbopogon citratus* in Malaria. *Journal of Protozoological Research* 3: 40–45

Paula, A.F.M. and Mark, A.B. (2011). Kidney function test. Retrieved on 2 March 2011 from www.surgeryencyclopedia.com

Pfeifer, A. and Neumann, H.G. (1986). Organ specific acute toxicity of the carcinogen *trans4*-acetylaminostilbene is not correlated with macromolecular binding. *Chemico-Biological Interactions* 59: 185–201

Philipson, J.D. (1993). Global trend and market size of herbal medicine in primary health care. In: *Trends in traditional medicine research* (Chan, K.L., ed.), p.1–8. Proceedings of International Conference in the use of traditional medicine and other natural products in health care, 8–11th June 1993, Penang, Malaysia. Universiti Sains Malaysia, Pulau Pinang.

Sommer, A.K. (2006). Fresh lemon grass fields in Israel become mecca for cancer patients? Israel21c: Innovation News Service. Retrieved on 4 October 2006 from www.israel21c.org

Thapa, B.R., and Walia, A. (2007). Liver function test and their interpretation. *Indian Journal of Pediatrics* 74: 67–75

Viana, G.S.B., Vale, T.G., Pinho, R.S.N. and Matos, F.J.A. (2000). Antinociceptive effect of the essential oil from *Cymbopogon citrates* in mice. *Journal of Ethnopharmacology* 70: 323–327

WHO (2003). Research guidelines for evaluating the safety and efficacy of herbal medicines. Geneva: World Health Organization.

Abstrak

Satu kajian telah dijalankan bagi menentukan kesan teh batang serai (*Cymbopogon citratus*) ke atas tikus betina *Sprague-Dawley*. Kajian ini bertujuan untuk membina satu garis panduan asas keselamatan menggunakan teh batang serai. Sejumlah 18 ekor tikus betina diberi minum teh batang serai pada dos 13 dan 130 mg/kg berat badan selama 28 hari. Tikus kawalan hanya diberi air suling. Darah tikus diambil untuk menilai fungsi ginjal dan hati. Pengambilan teh batang serai secara berulang tidak menyebabkan perubahan yang signifikan terhadap profil lipid darah tikus (kolesterol, lipoprotein ketumpatan tinggi (HDL), lipoprotein ketumpatan rendah (LDL) dan trigliserida), parameter fungsi hati [*aspartate transaminase* (AST), *alanine transaminase* (ALT) dan *gamma-glutamyl transferase* (GGT)], parameter fungsi buah pinggang (kreatinin, urea dan asid urik), jumlah protein dan kepekatan glukosa. Teh batang serai tidak menyebabkan toksik pada hati dan ginjal. Berat tikus yang meminum teh pada dos 130 mg/kg berat badan (10 kali lebih tinggi dos biasa manusia) didapati hampir sama dengan berat tikus kawalan. Kesemua tikus hidup sepanjang kajian tanpa menunjukkan apa-apa kesan kesakitan. Berdasarkan kajian ini, teh batang serai didapati tidak toksik walaupun pada kepekatan 10 kali lebih tinggi daripada kepekatan dos biasa manusia.

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