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RESEARCH ARTICLE

Acute and sub-chronic toxicity study of green coffee extract (*Coffea canephora L.*) on liver function of wistar rats

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Abstract

Background: Biodiversity is the main asset in traditional medicine development in Indonesia. Robusta green coffee has been widely studied for its activity, such as anti-hyperlipidemia, anti-obesity, and as a weight loss agent. Many products of green coffee extract are available on the market, but its toxicity data is still limited. This encourages toxicity tests to prove its safety, especially in the liver, the main organ in xenobiotic metabolism. **Objective:** To obtain acute and sub-chronic toxicity data of green coffee extract. **Method:** Robusta green coffee extract was obtained by the maceration method using 96% ethanol. Acute and sub-chronic toxicity testing refers to the OECD 432 and 407 methods. Rats were treated with green coffee extract at various dosages using oral gavage (2000 and 5000 mg/kg BW for acute dose and 250, 500, and 1000 mg/kg BW for sub-chronic toxicity test). In the acute toxicity study, green coffee extract was given once only. Observation was carried out for the first 24 hours until 14 days after, while treatment and observation in the sub-chronic toxicity test were conducted everyday for 28 days. Data collected in this study included number of deaths, physical condition, SGOT/SGPT level, gross morphology, and liver histopathology. **Result:** Green coffee extract had an LD_{50} cut-off of 2000 mg/kg BW which was categorised as a mild toxicity level. No liver function parameter changes were observed in acute and sub-chronic toxicity tests. **Conclusion:** Green coffee extract is safe and can be further developed in herbal dosage forms.

Introduction

Indonesia has the third largest biodiversity in the world to support the development of herbal-based medicines. Robusta coffee is widely cultivated in Indonesia and is a popular drink for people worldwide. Based on the manufacturing process, coffee is divided into black and green. Green coffee does not undergo a roasting process like black coffee and has a higher chlorogenic acid content than black coffee. The products are famous in Indonesia and were claimed to be weight loss agents. Several preclinical and clinical studies showed that green coffee plays a role in weight loss and has anti-hyperlipidemia activities (Samadi *et al.*, 2015; Christianty *et al.*, 2021).

According to the Minister of Health Regulation, to obtain a distribution permit for traditional medicines, toxicity tests are required to assess their safety. So far, there is no data regarding the toxicity of green coffee extract, either acute or sub-chronic. However, several chemical components in coffee may contribute to toxic effects at excess doses. The three main compounds are chlorogenic acid, caffeine, and trigonelline. LD_{50} oral chlorogenic acid is more than 1g/kg BW in immature rats, whereas LD_{50} caffeine is 367 mg/kg BW in albino rats (Meng *et al.*, 2014; Adamson, 2016). Caffeine was reported to increase ALT/ALP levels in rats and aggravate acute liver inflammation (Ohta *et al.*, 2007; Emmanuel *et al.*, 2017). The LD_{50} of trigonelline is 5000 mg/kg BW in rats. Among the three components, only caffeine has the potential for toxicity, especially in the

liver, where the drug is metabolised. However, the caffeine content in green coffee is only around 1.25%-2.5% (Patay *et al.*, 2016). Therefore, it can be estimated that green coffee extract (GCE) has very low toxicity, and it is safe to use as herbal medicine. This study tested green coffee extract's acute and sub-chronic toxicity to obtain scientific evidence regarding its safety, especially in the liver.

Method

Material

Green bean coffee was purchased from KSU Buah Ketakasi, Jember city. The materials used included ethanol 96%, aqua-distillate, CMC Na, HCl, NaCl, NH_4OH 28%, formaldehyde, ether, reagent for ALT/AST and creatinine, hematoxylin-eosin, entellan, and xylol. The experimental animals were male and female Wistar rats, healthy, 150-200g in weight, and about two to three months old.

Experimental design

The GCE was made by maceration method using ethanol 96% as a solvent. The extract suspension was prepared at doses of 250, 500, 1000, 2000, and 5000 mg/kg BW. The experimental procedure had been approved by the Ethical Committee of Medical Research, Faculty of Dentistry, Universitas Jember, with document number 1002/UN25.8/KEPK/DL/2020.

Mice were acclimatised for seven days and then prepared for an acute toxicity test referring to the OECD 423 method (OECD, 2002). Three rats received a GCE of 2000 mg/kg BW orally; then, the number of deaths in 24 hours and signs of toxicity up to 14 days is observed. If two to three test animals died, the dose would be reduced to 300 mg/kg BW; if zero to one test animal died, the dose of 2000 mg/kg BW would be repeated; if there were no death, the dose would be increased to 5,000 mg/kg BW in three different rats until the LD_{50} cut off value was found. Blood was drawn on 24 hours, the seventh, and the forteenth days to examine ALT/AST levels and the rats were sacrificed for liver preparations. The sub-chronic toxicity test refers to the OECD 470 method (OECD, 2008). Ten rats in each group were administered GCE with doses of 250, 500, and 1000 mg/kg BW. The treatment was given orally everyday for 28 days. The next day, blood was taken intra-cardiac for ALT/AST examination. The surgery was carried out by taking the liver to make Hematoxylin-Eosin (HE) preparations.

Statistical analysis

The results of AST/ALT level and organ relative weight data were presented as means ± standard deviation ($\bar{x}$ ± SD). In the acute toxicity test, t-test analysis was used to compare AST/ALT level and liver relative weight between control and GCE 2000 mg/kg BW groups. In the sub-chronic toxicity test, between-group comparisons were performed using a one-way analysis of variance (ANOVA), followed by LSD's procedure for multiple-range tests. Non-parametric data were analysed with Kruskal-Wallis, continued with the Mann-Whitney test. A value of $p < 0.05$ was considered significant.

Result

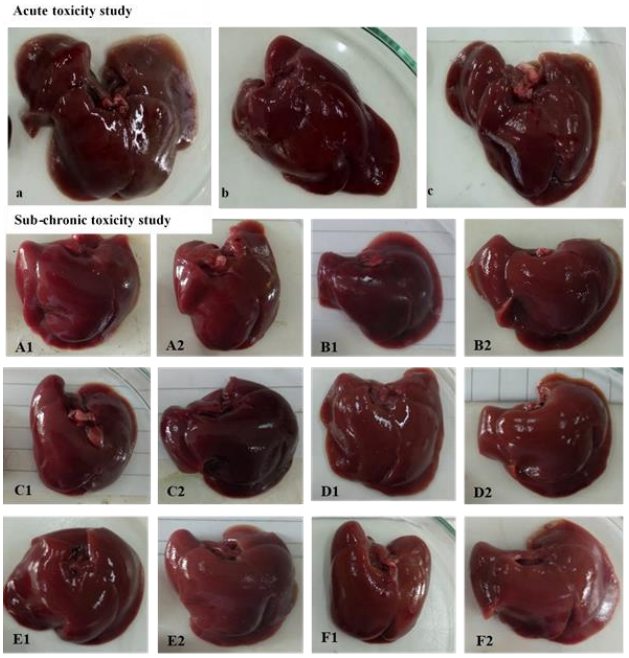
Acute toxicity study

Administration of GCE 2000 mg/kg BW did not cause death within 24 hours, and there were no signs of physical toxicity. The rats were active and had normal activities for up to 14 days of observation. The results of the second treatment with the same dose showed similar results. When the dose was increased to 5000 mg/kg BW, the first rat showed symptoms of weakness after five hours of treatment but became active after seven hours. The second rat died after five hours of administration. The third rat experienced weakness, seizures, and fast heart rate after five hours of treatment, and then died in the next hour. Based on the results, the LD_{50} cut-off value is more than 2000 mg/kg BW.

Based on a weekly analysis of AST/ALT levels and the percentage of liver relative weight, using an independent t-test, there was no significant difference between the control and treatment groups (Table I). No damage or lesions were found on the macroscopic liver, as shown in Figure 1.

Sub-chronic toxicity study

The statistical analysis of AST level, relative liver weight percentages using Kruskal-Wallis, and ALT levels using one-way ANOVA showed significant differences between several groups in male but not female rats. However, the result of the post hoc test showed significant differences occurred between the GCE treatment groups and the satellite groups, while the GCE treatment groups compared to the control group were not significantly different (Table II). It was supported by gross liver morphology observations in all groups, which also showed normal liver, fresh red colour, smooth texture, chewy consistency, and no fatty liver (Figure 1).



a = control, b = GCE 2000 mg/kg BW 1st dose, c = GCE 2000 mg/kg BW 2nd dose, A = control, B = GCE 250 mg/kg BW, C = GCE 500 mg/kg BW, D = GCE 1000 mg/kg BW, E = satellite control, F = satellite GCE 1000 mg/kg BW; 1 = male, 2 = female

Figure 1: Macroscopic rat liver results of acute and sub-chronic toxicity test of green coffee extract (GCE)

Table I: Parameters of liver function in acute toxicity study of green coffee extract

Parameters of liver function	Days	Groups		*p*-value†
		Control	GCE 2000	
AST (U/L)	1	208.03 ± 62.28	172.50 ± 47.89	0.370
	7	192.32 ± 39.55	163.83 ± 51.13	0.430
	14	134.73 ± 22.62	175.47 ± 48.46	0.219
ALT (U/L)	1	78.22 ± 29.05	71.95 ± 18.75	0.701
	7	69.98 ± 6.084	74.36 ± 10.61	0.536
	14	71.30 ± 6.996	63.27 ± 12.99	0.360
Liver relative weight (%)	14	4.04 ± 0.44	3.92 ± 0.12	0.533

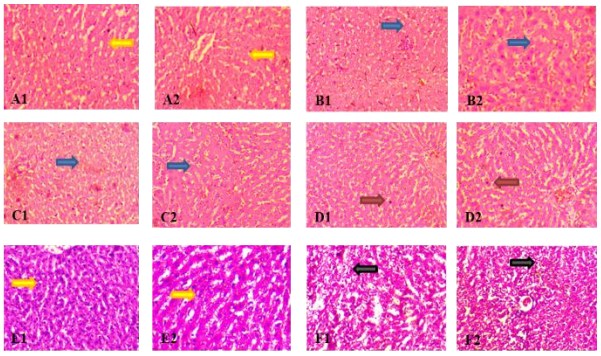
†Independent t-test, GCE = Green coffee extract

Table II: Parameters of liver function in sub-chronic toxicity study of green coffee extract

Parameters of liver function	Groups						*p*-value
	Control	GCE 250	GCE 500	GCE 1000	Satelite control	Satelite GCE 1000	
AST (U/L)							
Jantan	164±37^{a}	170±9^{a}	161±18^{a}	150±21^{ab}	138±15^{ab}	123±12^{b}	0.010†
Betina	170±13	177±24	160±18	140±23	137±17	121±48	0.052
ALT (U/L)							
Jantan	74±13^{a}	76±15^{a}	90±13^{a}	73±16^{ab}	64±10^{ab}	63±8^{ab}	0.032#
Betina	69±11	63±6	84±46	68±20	66±5	65±17	0.957
Liver relative weight (%)							
Jantan	3.17±0.53^{a}	3.24±0.27^{ab}	3.84±0.32^{ac}	3.47±0.27^{ad}	3.30±0.27^{ae}	3.11±0.12^{ae}	0.038†
Betina	3.46±0.28	3.86±1.1	4.05±0.34	3.50±0.41	3.89±0.39	3.87±0.45	0.056

†Kruskal-Wallis followed by Mann-Whitney tests, and #one-way ANOVA followed by LSD test. Different superscript letters indicate that there are significant differences between groups (p<0.05). GCE = Green coffee extract

The observation of liver histopathology showed that the liver structure generally looked normal, the hepatocyte cells were arranged tightly and regularly, and the sinusoid width was normal (Figure 2). Cell degeneration was found in the GCE 250 and 500 mg/kg BW groups, whereas cell apoptosis was performed in the GCE 1000 mg/kg BW group. Necrosis only appeared in the GCE 1000 mg/kg BW satellite group. Although cell damage was seen in some preparations, it was only found in a group of cells in one field and did not spread widely. Hence, it was suspected that it was not caused by GCE treatment.



Normal hepatocytes (→), hepatocyte degeneration (→), hepatocyte apoptosis (→), hepatocyte necrosis (→), A= control, B= GCE 250 mg/kg BW, C = GCE 500 mg/kg BW, D = GCE 1000 mg/kg BW, E = control satellite, F= GCE satellite 1000 mg/kg BW; 1 = male, 2 = female.

Figure 2: Histopathological picture of rat liver in sub-chronic toxicity study of green coffee extract (GCE), a cross-section with hematoxylin-eosin staining, magnification 400x

Discussion

No deaths and signs of toxicity were observed in GCE 2000 mg/kg BW treatment group. The LD_{50} cut-off value of GCE is > 2000 mg/kg BW (category V based on the GHS classification in the OECD). The extract had mild toxicity (LD_{50} between 500-5000 mg) according to the criteria for traditional medicines and other ingredients (BPOM, 2014). This is in line with previous acute toxicity studies of several coffee variants: black coffee bean powder and green coffee bean oil extract (Francis & Amonkan, 2016; Oliveira *et al.*, 2020). Signs of toxicity and death of rats in the GCE 5000 mg/kg BW treatment were estimated due to an excessive dose of caffeine contained in green coffee beans. Acute caffeine consumption stimulates a slight increase in blood pressure, bradycardia, or tachycardia, leading to cardiac arrhythmias. Deaths due to caffeine overdose have been reported with symptoms of tachyarrhythmias (Temple *et al.*, 2017). Chlorogenic acid, as the main content of green coffee beans, has low toxicity and side effects, and there are no studies or reports related to poisoning with high doses.

AST/ALT levels are important biochemical parameters as a marker of hepatotoxicity due to high exposure to xenobiotics in the liver and the detoxification role of the liver in the body. In the sub-chronic toxicity test, the AST/ALT levels of male rats showed significant differences in several groups, but these changes may not be caused by GCE administration. AST levels still meet the normal range of male and female rats (60-300 IU/L and 80-250 IU/L), while the ALT value is higher than the standard value, which is 25-55 IU/L in males and 25-50 IU/L in females (Sharp & Villano, 2012). Various factors, including stress, strongly influence changes in both levels. Stress can be caused by physical factors, especially during handling, treatment, and environmental conditions. Stress can affect all organs, including the liver. According to Jia and researchers, mild stress that lasts for a long time can change the liver's metabolic profile (Jia *et al.*, 2016). Under stressful conditions, the immune tolerance of the liver would be impaired, resulting in liver inflammation. High norepinephrine from excessive stress hormone production and activation of the sympathetic nervous system will activate Kupffer cells and produce excess free radicals, thereby exacerbating liver inflammation (Joung *et al.*, 2019).

Liver toxicity can also be determined based on changes in organ weights and observing the macroscopic or microscopic images of the liver. Although there were significant differences in several groups of male rats, it was thought that it was not caused by the administration of GCE. It was supported by gross morphological and histopathological data of the liver, which showed that both the macroscopic and microscopic liver was normal. Some structural damages that appear on the histopathological picture of the liver, such as cell degeneration and apoptosis, are a normal phenomenon that occurs in living things. If cell degeneration and apoptosis continue to spread to large tissues, it can cause necrosis and permanent tissue damage.

In general, GCE does not cause hepatotoxicity. Although some studies have shown that caffeine can increase liver toxicity, the deficient levels in GCE can be masked by the effects of other components, such as chlorogenic acid, which has a hepatoprotective effect. Research showed that chlorogenic acid could lower the ALT value of triptolide-induced mice, protecting the mice from LPS-induced hepatotoxicity, and protecting liver tissue by inhibiting inflammatory reactions through toll-like receptor signalling mechanisms. It also strengthens the antioxidant defence system through free radical scavenging (Wang *et al.*, 2018; Miao &

Xiang, 2020). Few studies have been conducted to study the toxicity of trigonelline compounds. The administration of 50 mg/kg of trigonelline to rats orally every day for 21 days for rats does not have an effect on liver weight (Zhou *et al.*, 2012). Trigonelline also exists in a smaller amount compared to caffeine and chlorogenic acid (0.7:2.5:11.5), so the effect of the compound is not visible (Chu, 2012).

Conclusion

The green coffee extract has mild toxicity and does not cause hepatotoxicity either in acute or sub-chronic administration, making it possible to develop herbal products.

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Reference

Adamson, R. H. (2016). The acute lethal dose 50 (LD50) of caffeine in albino rats. *Regulatory Toxicology and Pharmacology*, *80*, 274–276. https://doi.org/10.1016/j.yrtph.2016.07.011

BPOM. (2014). Peraturan Kepala Badan Pengawas Obat Dan Makanan Republik Indonesia Nomor 7 Tahun 2014. *Tentang Pedoman Uji Toksisitas Nonklinik Secara in Vivo*, 66–68. https://doi.org/10.1017/CBO9781107415324.004

Christianty, F. M., Fajrin, F. A., & Roni, A. (2021). The potential effect of the green coffee extract on reducing atherogenic index in hyperlipidemic rats. *Pharmacy Education*, ***21***(2), 126–131. https://doi.org/10.46542/pe.2021.212.126131

Chu, Y. F. (2012). Coffee: Emerging Health Effects and Disease Prevention. *Coffee: Emerging Health Effects and Disease Prevention*. https://doi.org/10.1002/9781119949893

Emmanuel, A., Majesty, D., Benjamin, A., Peter, A., & Princess, U. (2017). Effect of Caffeine on Some Selected Biochemical Parameters Using Rat Model. *Advances in Biology*, *2017*, 1–8. https://doi.org/10.1155/2017/9303276

Francis, A., & Amonkan, A. (2016). Acute toxicity of the aqueous extract of roasted and ground beans of Coffea canephora robusta in the wistar rat. *The Pharma Innovation Journal*, ***5***(12), 1–5. www.thepharmajournal.com

Jia, H.-M., Li, Q., Zhou, C., Yu, M., Yang, Y., Zhang, H.-W., Ding, G., Shang, H., & Zou, Z.-M. (2016). Chronic unpredictive mild stress leads to altered hepatic metabolic profile and gene expression. *Scientific Reports*, *6*, 23441. https://doi.org/10.1038/srep23441

Joung, J.-Y., Cho, J.-H., Kim, Y.-H., Choi, S.-H., & Son, C.-G. (2019). A literature review for the mechanisms of stress-induced liver injury. *Brain and Behavior*, ***9***(3), e01235. https://doi.org/10.1002/brb3.1235

Meng, Z. Q., Tang, Z. H., Yan, Y. X., Guo, C. R., Cao, L., Ding, G., Huang, W. Z., Wang, Z. Z., Wang, K. D. G., Xiao, W., & Yang, Z. L. (2014). Study on the anti-gout activity of chlorogenic acid: Improvement on hyperuricemia and gouty inflammation. *American Journal of Chinese Medicine*, ***42***(6), 1471–1483. https://doi.org/10.1142/S0192415X1450092X

Miao, M., & Xiang, L. (2020). Pharmacological action and potential targets of chlorogenic acid. *Advances in Pharmacology (San Diego, Calif.)*, *87*, 71–88. https://doi.org/10.1016/bs.apha.2019.12.002

OECD. (2002). Test No. 423: Acute Oral toxicity - Acute Toxic Class Method. *Oecd Guideline for Testing of Chemicals*, *December*, 1–14. https://doi.org/10.1787/9789264071001-en

OECD. (2008). OECD Guidelines for the testing of chemicals: Repeated Dose 28-day Oral Toxicity Study in Rodents. *Drug and Chemical Toxicology*, ***34***(1), 13. https://doi.org/10.1787/9789264070684-en

Ohta, A., Lukashev, D., Jackson, E. K., Fredholm, B. B., & Sitkovsky, M. (2007). 1,3,7-trimethylxanthine (caffeine) may exacerbate acute inflammatory liver injury by weakening the physiological immunosuppressive mechanism. *Journal of Immunology (Baltimore, Md. : 1950)*, ***179***(11), 7431–7438. https://doi.org/10.4049/jimmunol.179.11.7431

Oliveira, N. A. de, Sandini, T. M., Cornelio-Santiago, H. P., Martinelli, E. C. L., Raspantini, L. E. R., Raspantini, P. C., Momo, C., Oliveira, A. L. de, & Fukumasu, H. (2020). Acute and subacute (28 days) toxicity of green coffee oil enriched with diterpenes cafestol and kahweol in rats. *Regulatory Toxicology and Pharmacology*, 110(October 2019), 104517. https://doi.org/10.1016/j.yrtph.2019.104517

Patay, É. B., Bencsik, T., & Papp, N. (2016). Phytochemical overview and medicinal importance of Coffea species from the past until now. *Asian Pacific Journal of Tropical Medicine*, ***9***(12), 1127–1135. https://doi.org/10.1016/j.apjtm.2016.11.008

Samadi, M., Mohammadshahi, M., & Haidari, F. (2015). Green Coffee Bean Extract as a Weight Loss Supplement. *Nutritional Disorder & Therapy*, ***5***(4), 1–3. https://doi.org/10.4172/2161

Sharp, P., & Villano, J. (2012). The laboratory rat, second edition. In *The Laboratory Rat, Second Edition: Vol. i*. https://doi.org/10.1201/b13862

Temple, J. L., Bernard, C., Lipshultz, S. E., Czachor, J. D., Westphal, J. A., & Mestre, M. A. (2017). The Safety of Ingested Caffeine: A Comprehensive Review. *Frontiers in Psychiatry*, *8*, 80. https://doi.org/10.3389/fpsyt.2017.00080

Wang, J.-M., Chen, R.-X., Zhang, L.-L., Ding, N.-N., Liu, C., Cui, Y., & Cheng, Y.-X. (2018). In vivo protective effects of chlorogenic acid against triptolide-induced hepatotoxicity and its mechanism. *Pharmaceutical Biology*, ***56***(1), 626–631. https://doi.org/10.1080/13880209.2018.1527370

Zhou, J., Chan, L., & Zhou, S. (2012). Trigonelline: a plant alkaloid with therapeutic potential for diabetes and central nervous system disease. *Current Medicinal Chemistry*, ***19***(21), 3523–3531. https://doi.org/10.2174/092986712801323171