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A cytotoxicity and sub-acute toxicity study on tea leaves cultivated in Sabah

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Abstract

The present work investigated the cytotoxicity capacity of the MDA-MB-231 (human cancer-derived), A549 (human lung cancer-derived), Caov3 (human ovarian cancer-derived), and HeLa (human cervical cancer-derived) cell lines on a wide range of tea leaves; green tea, black tea, tea waste, and compost from Sabah. A group of male and female Sprague Dawley rats was used to screen the sub-acute toxicity of green tea extract in tea leaves from Sabah for 28 d. Results revealed that the ethanol extract of tea leaves had strong cytotoxic activity against all cancer lines. Tea waste showed higher cytotoxicity when extracted using hot water. The ethanol extract of black tea leaves exhibited the highest inhibitory activity against the proliferation of Caov3, whereas the ethanol extract of green tea leaves exhibited a promising cytotoxic activity against MDA-MB-231 and HeLa cell lines. Toxicity studies showed decreased testes weight and increased liver weight in male rats that were administered with 5000 mg/kg of tea extract. This coincided with the significant increase portrayed by enzyme alanine aminotransferase (ALT) in the serum of treated male rats in the 5000 mg/kg dose group. Moreover, there was an increase of alkaline phosphatase (ALP) and ALT for the female rats in the 5000 mg/kg dose group. The increased levels of ALT and ALP enzymes, as well as liver weight, signified mechanical trauma in the liver of male and female rats in the 5000 mg/kg dose group.

Introduction

Tea (*Camellia sinensis*) is one of the most popular and widely consumed beverages worldwide, and it has many health benefits for humans. Tea is classified into two types. The first type is green tea which is a non-fermented drink widely consumed in China and Japan. The second type is black tea which is more common in North America and Europe. Green tea is now one of the most popular beverages in Malaysia, and its consumption is growing due to its fine taste and beneficial health effects. Green tea is a potent weapon against cancer cells (Pandey *et al.,* 2010). It reduces cardiovascular diseases and cholesterol, and induces body weight loss. These are mostly contributed by the presence of polyphenols in tea leaves such as (-)-epicatechin gallate (ECG), (-)-epicatechin (EC), (-)-epigallocatechin 3-gallate (EGCG), (-)-epigallocatechin (EGC), theaflavins, and thearubigins (Henning *et al.,* 2004; Deka and Vita, 2011).

Theaflavins and thearubigins are oligomeric polyphenolic compounds synthesised from monomeric tea flavanol units. The epidemiological studies suggested that green tea may prevent the development and progression of prostate carcinoma in comparison to black tea (Henning *et al.,* 2004). Tea is usually processed from young tea leaves, while mature and old ones become agricultural waste, and are often used as compost and animal feed. Although not many studies probed into mature and old tea leaves, Nor Qhairul Izzreen and Mohd Fadzelly (2013) found that tea leaves compost had a relatively similar amount of antioxidant activity in comparison to the green and black tea leaves when they were evaluated via FRAP assay. The assessment revealed that the compost contained 13.67 ± 0.21 µmol Fe2SO4•7H_2O/mL of antioxidant activity, followed by 14.00 ± 0.10 µmol Fe2SO4•7H_2O/mL for young black tea, and 14.03 ± 0.21 µmol Fe2SO4•7H_2O/mL of antioxidant activity for young green tea leaves.

More recent work also highlighted that the contents of polyphenol (-)-epigallocatechin in old

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(72.60 ± 0.07 mg/g), mature (70.64 ± 0.57 mg/g), and young (70.27 ± 0.10 mg/g) green leaves were relatively similar (Nor Qhairul Izzreen *et al.,* 2019). Farhoosh *et al.* (2007) and Nor Qhairul Izzreen and Mohd Fadzelly (2013) asserted that old tea leaves could still contribute to the antioxidant activity of tea. Tea polyphenols could also inhibit tumour genesis and tumour progression at different organ sites, in different animal models. The major active constituents in green tea are catechins. Epigallocatechin-3-gallate (EGCG) is the most abundant active compound and is considered as a promising cancer preventive agent, which helps inhibit the development of some diseases such as angiogenesis, osteogenesis, and prostate cancer (Davalli *et al.,* 2012; Chu *et al.,* 2017). However, these polyphenols also cause certain detrimental effects on human health such as the disruption of kidney functions and hepatotoxicity in mice (Lambert *et al.,* 2010) when it is consumed more than 1500 mg/kg of the weight of the mice.

Certain varieties of tea can elicit severe allergic reactions such as hay fever, asthma, nausea, and vomiting in patients, and the severity depends on the types of tea consumed. The toxicity from green and black tea occurs only at high doses. Caffeine in green tea can cause hypertension and osteoporosis because the LD_{50} dose of caffeine in green tea is estimated to be around 10 - 14 g (150 - 200 mg/kg of green tea) (Henning *et al.,* 2004). Besides caffeine, the presence of xanthine in tea is also responsible for a variety of toxic effects including nervousness, irritability, convulsions, tachycardia, and gastric irritation (Jain *et al.,* 2013). The hepatotoxic effects of different components of green tea *in vitro* had been reported. In this case, the amount of toxicity in the liver cells of rats was observed with high concentrations of hydro-alcoholic green tea extracts (100 - 500 g/mL) and eventually, EGCG was responsible for this effect (Schmidt *et al.,* 2005).

Black tea has a high concentration of tannins up to 0.8 mg/mL (Pasha and Reddy, 2005). This inhibited non-haem iron via the formation of insoluble complexes with ferric iron, which eventually affected the absorption of iron in the lumen (Jain *et al.,* 2013). Since green tea and black tea only exhibit effect at high doses, their consumption should not be allowed to exceed beyond a certain level. Although many studies had extensively examined the health benefits associated with the different varieties of tea, as well as their cytotoxicity activity, however, until today, studies on cytotoxicity and toxicity of tea leaves planted only in Sabah had rarely been reported. In addition, report on the potential of matured and old leaves that are usually being discarded into poultry feed or waste tea leaves is still limited. Therefore, the present work evaluated the cytotoxicity capacity and sub-acute toxicity of Sabah tea leaves at different maturity levels and fermentation stages, namely green tea, black tea, and black tea compost. Then, cytotoxicity studies were evaluated against the MDA-MB-231 (human cancer-derived), A549 (human lung cancer-derived), Caov3 (human ovarian cancer-derived), and HeLa (human cervical cancer-derived) cell lines. Finally, the sub-acute toxicity test was utilised to examine male and female Sprague Dawley rats for 28 d.

Materials and methods

Sample

The Sabah Tea Plantation supplied the tea leaves analysed in the present work.

Sample preparation

Tea leaves were prepared following the method suggested by Nor Qhairul Izzreen and Mohd Fadzelly (2013). Green tea leaves were freshly picked and steam-blanched for 10 min to deactivate enzymes in the leaves. These leaves were then kept in the oven at 25°C for 24 h, and grounded into smaller particles via MX-M200WSL Panasonic grinder for an additional 5 s. Next was the fermentation process of the black tea. The black tea leaves were put into an air-tight container and steamed at 40°C for 5 h. The leaves were dried in an oven for 10 min at 100°C, followed by 10 min at 90°C, 10 min at 60°C, and finally 10 min at 40°C until the moisture content was reduced to approximately 5%. The tea compost was derived from black tea, which had gone through a fermentation process, however, at the end of the grading process, it could not be graded with other tea leaves and was, therefore, separated to become compost. Compost consists of broken pieces of tea leaves that are usually sold for feedstock. These tea leaves were then ground into smaller particles.

Sample extraction for cytotoxicity studies

For water extraction, 5 g of tea powder was diluted and extracted with 50 mL hot water at 70°C, and kept for 24 h. The sample was then filtered with Whatman filter paper no. 4, and evaporated at 40°C. The extract was then referred to as hot water extract.

For ethanol extraction, the tea powder was extracted with ethanol in the ratio of 1:5 (w/v). About 100 g of powdered freeze-dried sample was soaked in 500 mL ethanol for three times overnight. Each infusion was filtered, and the three filtrates were combined and concentrated using a rotary evaporator. The concentrated extracts were diluted using dimethyl

sulfoxide to a concentration of 10 mg/mL.

MTT (*3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide*) *assay*

The MDA-MB-231 (human cancer-derived), A549 (human lung cancer-derived), Caov3 (human ovarian cancer-derived), and HeLa (human cervical cancer-derived) cell lines were grown in the RPMI 1640 medium growth in cell cultures, supplemented with 10% foetal bovine serum, 100 IU/mL of penicillin, and 100 µg/mL streptomycin at 37°C under 5% CO_2 in a humidified atmosphere. The viability of cells was determined by staining with trypan blue. The log phase of growing cells was harvested and counted by using a haemocytometer. The cells were diluted in a culture medium to a concentration of 1 x 10^5 cells/mL From this cell suspension, 100 µL was pipetted into each well of a 96-well microtiter plate and incubated for 24 h in a 5% CO_2 incubator at 37°C. The old medium was tapped out and sample extracts (diluted in medium) in the range of 10, 20, 40, 60, 80, and 100 µg/mL were added into the plate. The plate was incubated in a 5% CO_2 incubator at 37°C for 72 h. Then, 10 µL of MTT reagent (Roche, USA) was added into each well. This plate was incubated again for 4 h in a CO2 incubator at 37°C. Subsequently, 100 µL of solubilisation solution (Roche, USA) was added into each well. The cells were then left overnight at 37°C in a CO_2 incubator. Finally, the absorbance was read using a microplate reader (at a wavelength of 550 nm). Cytotoxicity (%) was calculated using Eq. 1:

% cytotoxicity = (optical density of sample / optical density of control) × 100 (Eq. 1)

The median inhibition concentration (IC_{50}) or the concentration of extract, which was able to inhibit cell proliferation by 50%, was calculated graphically for each cell proliferation curve.

Experimental design for toxicity studies

Groups of 20 male and 20 female Sprague Dawley rats were administered with 0, 125, 250, and 500 mg/mL of green tea extract in de-ionised water by gavage for 5 d a week, for a total of 28 d. The total dosing volume was 2.7 mL/kg body weight for male rats, and 1.4 mL/kg for female rats. Ten additional male and female rats per dose group were included for haematology and clinical chemistry analyses. These animals were sacrificed on day 23 (23-d study groups). Animals were observed twice a day for signs of mortality or morbidity. Individual animal weights were recorded weekly. At the end of day-28, animals fasted, and on day-29, the rats were euthanised by CO_2 asphyxiation. Animals were anesthetised with a CO_2/air mixture, and blood was obtained from the retro-orbital plexus. Finally, the blood samples for haematology were collected into serum separator tubes for clinical chemistry analyses.

Animal and housing

These studies were conducted following the United States Public Health Service policy on humane care and the use of laboratory animals, and the Guide for the Care and Use of Laboratory Animals (National Research Council, 1996). Additionally, these studies were conducted in compliance with the Good Laboratory Practice Regulations (Food and Drug Administration, 1987). Twenty male and female Sprague Dawley rats (20 to 40 d old) were obtained from the veterinary department in Universiti Putra Malaysia, and quarantined for 10 d before dosing. Water and feed (Cargill, Malaysia) were available *ad libitum*. The room temperature was kept at 23 - 25°C, and the humidity was kept at 35 - 65% throughout the study. The fluorescent light cycle was 12 h per day. The rats and mice were randomly assigned to treatment groups. Male mice were housed individually, and rats and female mice were housed 5 per cage, in polycarbonate cages containing Sani-Chips bedding from P.J. Murphy Forest Products Corporation (Montville, NJ).

Body and organ weights

Total body weight and weights of the liver, thymus, right kidney, right testis, heart, lungs, and spleen were determined at terminal sacrifice.

Clinical chemistry

Blood samples from each animal were analysed for the clinical chemistry endpoints [i.e., glucose, alanine aminotransferase (ALT), alkaline phosphatase (ALP), urea nitrogen, creatinine, and creatine kinase (CK)], and were analysed using the Hitachi 911 (Roche, Indianapolis, IN).

Statistical analysis

All experiments were carried out in triplicate and presented as mean ± standard deviation (SD) using SPSS version 15.0. The data were statistically analysed by one-way ANOVA. When ANOVA showed significant differences, *post-hoc* Tukey-test was computed (95% significance level) to describe the effects of the parameter tested.

Results and discussions

Cytotoxicity studies

MTT assay is a widely utilised method in the study of cytotoxicity of natural products. This assay is based on the ratio of yellow tetrazolium MTT salt cleavage to purple formazan crystal in the mitochondria of living cells (Mosmann, 1983). The absorbance of solubilised formazan crystal is equal to the number of living cells in the system. The present work had initially examined the crude extracts of Sabah tea prior to the different sets of treatment (fresh, fermented, compost, and waste) to determine their cytotoxicity against the MDA-MB-231 (human cancer-derived), A549 (human lung cancer-derived), Caov3 (human ovarian cancer-derived), and HeLa (human cervical cancer-derived) *in vitro* (Table 1).

Table 1 shows that ethanol extract of tea leaves induced a strong cytotoxic activity against almost all cancer cell lines that were tested, except for waste tea leaves. Interestingly, waste tea leaves possessed higher cytotoxic activity because the values were relatively lower than ethanol extractions. Therefore, the lower the value of IC_{50}, the more effective is the anti-cancer potential of a pure compound or crude extract. The ethanol extract of black tea leaves exhibited an IC_{50} value of 15, which signified the highest inhibitory activity against the proliferation of Caov3. Green tea leaves showed potent cytotoxic activity against the MDA-MB-231 cell lines when the hot water signified an IC_{50} value of 34, and an ethanol extraction with an inhibitory concentration of 30. However, hot water green tea extraction possessed the lowest inhibitory concentration of 30 against the HeLa cell line, with zero cytotoxic activity for ethanol extraction. Hot water and ethanol extraction for all types of tea leaves showed a favourable cytotoxic activity against the MDA-MB-231 cell lines. Hot water extraction exhibited an IC_{50} value between 34 and 95, whereas the ethanol extraction displayed an IC_{50} value between 65 and 92. Tea compost and tea waste showed potential inhibitory activity against the proliferation of all cell lines except the HeLa cell because the values of IC_{50} were between 39 and 88, and 63 and 95, respectively.

Table 1 also shows that black tea leaves in the A549 and HeLa cancer cell lines showed zero cytotoxic activity. Green and black tea had proved to possess anti-cancer properties because of their polyphenol content such as catechin-3-gallate and epigallocatechin-3-gallate in breast cancer cell, bladder, lung, prostate, and human bronchial cell lines (Asensi *et al.,* 2011). Phytochemicals such as phenolic acids and flavonoids were responsible for anti-proliferative properties. The black and green Sabah tea contained a considerably high number of polyphenolic compounds that were concomitant with superior antioxidant properties (Nor Qhairul Izzreen and Mohd Fadzelly, 2013; Nor Qhairul Izzreen et al., 2019). The cytotoxic activity of extracts was contributed by a broad range of phenolic phytochemicals' pool based on the HPLC assessment, in a previous work.

Body and organ weights

Two female rats died after 28-d of the study. The cause of death was not related to treatment. The other animals survived until scheduled sacrifice. Therefore, it was concluded that this treatment had no

Table 1. IC_{50} values of tea extraction on different types of cancer cell lines.

Tea sample	A549	MDA -MB - 231	Caov3	HeLa
Green				
Hot water	nd	92.0 ± 2.90	nd	100.0 ± 7.40
Ethanol	61.0 ± 3.77	34.0 ± 4.40	85.0 ± 7.35	30.0 ± 0.50
Black				
Hot water	nd	90.0 ± 1.50	nd	nd
Ethanol	nd	37.0 ± 0.50	15.0 ± 0.50	40.0 ± 2.50
Compost				
Hot water	53.0 ± 4.31	65.0 ± 3.50	70.0 ± 2.50	nd
Ethanol	88.0 ± 3.51	39.0 ± 2.30	48.0 ± 2.00	nd
Waste				
Hot water	nd	68.0 ± 6.00	60.0 ± 4.00	nd
Ethanol	63.0 ± 4.70	95.0 ± 5.35	85.0 ± 4.50	nd

Data are mean ± standard deviation of three separate experiments. nd = not detected.

effects on survival. Table 2 summarises the effect of tea extract on the terminal body and on selected weight of the rats' organs in 28 d. The results revealed that total body weight-gain had decreased in both male and female rats from all dose groups. This stemmed from the consumption of green tea that contained tea polyphenol EGCG, which had previously been demonstrated in animals (William *et al.,* 2003; Chan *et al.,* 2010a). In the present work, male rats were more affected than female rats, which is consistent with William *et al.* (2003) and Chan *et al.* (2010a). The weight reduction also resulted from the changes in food consumption, inhibition of intestinal lipid absorption, an increase in the expenditure of energy, and stimulation of lipid oxidation (Rains *et al.,* 2011; Lee and Jia, 2015).

Hepatosomatic index (HSI) and gonadosomatic index (GSI) is another parameter of ecotoxicology, in which the calculation is based on the ratio of liver and gonad (sex gland) weight per body weight,

Table 2. Weights and selected organ weights of male and female Sprague Dawley rats administered with green tea extract by gavage for 28 d.

Parameter	Green tea extract (mg/kg)			
	0	125	250	500
Males				
Terminal body weight (g)	308.2 ± 14.94	283.7 ± 16.19	293.16 ± 14.32	266.84 ± 7.78
Organ weight (g)				
Kidney	2.52 ± 0.47	2.36 ± 0.34	2.28 ± 0.13	2.46 ± 0.30
Heart	1.12 ± 0.23	1.14 ± 0.11	1.20 ± 0.21	1.12 ± 0.11
Liver	9.50 ± 0.98	9.18 ± 1.00	9.60 ± 1.01	9.82 ± 0.72
Lung	2.02 ± 0.53	1.76 ± 0.32	1.92 ± 0.13	1.88 ± 0.24
Testis	3.24 ± 0.21	3.06 ± 0.21	2.94 ± 0.15	2.88 ± 0.19*
Organ to body weight ratio (mg organ weight/g body weight)				
Kidney	0.82 ± 0.14	0.83 ± 0.12	0.78 ± 0.04	0.92 ± 0.11
Heart	0.37 ± 0.07	0.40 ± 0.04	0.41 ± 0.07	0.42 ± 0.04
Liver	3.08 ± 0.32	3.24 ± 0.35	3.27 ± 0.35	3.68 ± 0.27*
Lung	0.65 ± 0.17	0.66 ± 0.17	0.65 ± 0.04	0.70 ± 0.09
Testis	1.05 ± 0.07	1.18 ± 0.07	1.00 ± 0.05	1.08 ± 0.07
Females				
Terminal body weight (g)	189.14 ± 25.00	181.54 ± 12.11	172.52 ± 9.54	164.02 ± 7.44
Organ weight (g)				
Kidney	1.44 ± 0.25	1.34 ± 0.11	1.52 ± 0.18	1.28 ± 0.04
Heart	0.72 ± 0.11	0.74 ± 0.05	0.78 ± 0.04	0.72 ± 0.08
Liver	7.22 ± 0.93	6.74 ± 0.47	7.14 ± 1.16	6.78 ± 0.34
Lung	1.42 ± 0.18	1.34 ± 0.29	1.46 ± 0.22	1.20 ± 0.10
Ovary	-	-	-	-
Organ to body weight ratio (mg organ weight/g body weight)				
Kidney	0.76 ± 0.13	0.74 ± 0.06	0.89 ± 0.11	0.78 ± 0.03
Heart	0.38 ± 0.05	0.41 ± 0.03	0.44 ± 0.02	0.44 ± 0.05
Liver	3.82 ± 0.49	3.72 ± 0.27	4.14 ± 0.68	4.13 ± 0.21
Lung	0.75 ± 0.10	0.74 ± 0.16	0.85 ± 0.13	0.73 ± 0.06
Ovary	-	-	-	-

Data are mean ± standard deviation. Ten animals were evaluated in each group. * = statistical significance at $p \leq 0.05$, (-) = too small to dissect.

respectively (Jelodar and Fazli, 2012). The increased HSI and GSI values and tested dosage indicated exposure to a toxic environment or contamination. In the present work, the decreased testes weight and increased liver weight in male rats administered with 5000 mg/kg tea extract were the only relatable treatment. There were no significant changes in body and organ weight for female dose groups. Hepatic toxicity in general signifies the increase or the decrease of liver weight. The liver weight of normal rats is 4% of the rats' total body weight. Although the liver weight had increased in comparison to the control, the ratio of liver weight to total body mass was still in the normal range.

The experiment revealed that there was an increase in the value of the HSI index among male rats that were administered with the highest dosage of tea extract (3.68 ± 0.27 mg/g) in comparison to the control group (3.08 ± 0.32 mg/g). Nevertheless, HIS index values for female rats showed no significant difference between the control group of rats (3.82 ± 0.49 mg/g) and the rats administered with 5000 mg/kg of tea extract (4.13 ± 0.21 mg/g). The increased liver weight observed in the present work resulted from acute or chronic liver injuries, and therefore, the catechins and their gallic acid esters, predominantly EGCG, were the main components responsible for the hepatotoxicity (Mazzanti *et al.,* 2009). Similarly, Chan *et al.* (2010b) observed liver necrosis in mice administered with 1000 mg/kg dose tea extract. However, the present work proposed that hepatotoxicity might have resulted from oxidative stress, which was induced in the liver by EGCG or its metabolites.

There was a decrease in the testes' weight of male rats that were administered with 5000 mg/kg of tea extract. Nevertheless, the weight of the testes to total body weight was still in the normal range. Additionally, no significant difference was observed in the values of GSI indexes between the control group (1.05 ± 0.07 mg/g) and the male rats that were administered with 5000 mg/kg of tea extract (1.08 ± 0.07 mg/g). However, the differences in weight might have resulted from the loss of germ cells and a decreasing amount of fluid from the tubule seminiferous, which reduced the diameter of the testes, its size, and weight (Creasy and Foster, 2002).

Clinical chemistry

The common enzymes that indicate hepatocellular damages are transaminase enzymes (aspartate aminotransferase, AST; alanine aminotransferase, ALT; and alkaline phosphatase, ALP). The measurement of ALT and AST enzymes' serum levels can be used to evaluate liver damage. Therefore, an increase in those enzymes in plasma may indicate liver damage (Ekam and Ebong, 2007). Table 3 shows that there was a significant increase in ALT in the volume of serum for treated male and female rats in the 5000 mg/kg dose groups, in comparison to the control group with the amount of 81.40 ± 10.06 and 71.80 ± 14.25 mg/kg, respectively. An increase in these enzymes might have resulted from the high consumption of tea extract, which subsequently affected the liver. Surprisingly, there was a decrease in the quantity of ALP, before and after treatments, in both the male and female rats' dose groups. Although both enzymes indicated a possible sign of liver damage, this was inconsistent with the amount of ALT observed. However, a slight increase of ALP was observed before (109.42 ± 57.30 mg/kg) and after (115.80 ± 12.24) treatment in female rats in the 5000 mg/kg dose group.

Nonetheless, minor liver damage might still occur because of the increased level of ALT and liver weight in male rats, even though the amount of ALP decreased. Similarly, the decreased content of the ALT and ALP blood serum enzymes in female rats suggested slight liver damage. Despite the inconsistency between ALT and ALP, results also suggested that there could be a mechanical trauma in the liver. Galati *et al.* (2006) and Mazzanti *et al.* (2009) claimed that tea polyphenols might cause a hepatoxicity risk in humans as a result of the pro-oxidant activities of potential toxic effects in normal hepatocyte and livers, in which the cytotoxicity effect depended on time and dose.

Blood urea nitrogen and serum creatinine typically signify the liver and kidney functions in a body, respectively. A high level of blood urea nitrogen and serum creatinine can also cause detoxification because both detoxification and the function of the liver and kidney were closely related. Urea and creatinine travel from liver to kidney via the bloodstream, and therefore, healthy kidneys would be able to filter and remove urea and creatinine from the blood. The present work observed increased levels of creatinine in male and female rats in all dose groups, specifically in the highest dose groups with the amount of 33.20 ± 3.70 and 35.20 ± 1.79 mg/kg, respectively. However, the difference was insignificant ($p > 0.05$) as compared to the control group. A similar trend was observed for the levels of urea, as an increased amount of urea was found in male and female rats in dose group 5000 mg/kg with levels of 8.40 ± 1.41 and 7.42 ± 0.51 mg/kg respectively (Table 3). There was no renal dysfunction because the level of both enzymes and the weight of the

Table 3. Biochemistry of clinical serum from male and female Sprague Dawley rats in 28-d gavage study with green tea extract.

Parameter		Green tea extract (mg/kg)			
		0	1250	2500	5000
Males					
ALT (IU/L)	Before	58.00 ± 10.98	61.2 ± 18.26	65.4 ± 23.17	62.00 ± 32.07
	After	55.80 ± 6.42	78.00 ± 8.89	65.00 ± 13.70	81.40 ±10.06*
ALP (IU/L)	Before	154.00 ± 35.76	181.40 ± 71.80	177.20 ± 50.71	169.40 ± 48.80
	After	158.00 ± 11.68	106.80 ± 8.56	116.40 ± 15.92	119.40 ± 19.88
CK (IU/L)	Before	463.16 ± 78.17	530.42 ± 20.50	673.15 ± 54.28	538.54 ± 65.25
	After	615.62 ± 25.93	651.26 ± 23.02	905.02 ± 80.22	769.72 ± 98.55
KREA (µmol/L)	Before	24.80 ± 4.90	27.00 ± 4.74	28.20 ± 4.87	27.20 ± 2.49
	After	32.80 ± 2.59	33.40 ± 4.83	36.20 ± 4.32	33.20 ± 3.70
GLU (mmol/L)	Before	7.12 ± 1.42	8.24 ± 1.38	8.32 ± 1.17	8.24 ± 0.26
	After	7.54 ± 0.80	7.74 ± 0.65	7.76 ± 1.24	6.16 ± 0.46*
UREA (mmol/L)	Before	6.66 ± 0.62	7.24 ± 0.45	7.60 ± 0.76	7.44 ± 1.09
	After	7.68 ± 0.81	6.68 ± 1.46	8.36 ± 1.01	8.40 ± 1.41
Females					
ALT (IU/L)	Before	51.40 ± 18.27	51.60 ± 23.35	48.00 ± 11.85	61.20 ± 16.63
	After	53.60 ± 2.41	57.60 ± 11.01	68.40 ± 6.58	71.80 ± 14.25*
ALP (IU/L)	Before	152.60 ± 55.36	148.60 ± 68.64	125.40 ± 41.73	109.42 ± 57.30
	After	150.20 ± 8.98	98.40 ± 4.83	110.80 ± 14.92	115.80 ± 12.24
CK (IU/L)	Before	453.66 ± 25.30	357.00 ± 70.41	333.46 ± 70.59	262.4 ± 94.95
	After	980.00 ± 38.31	635.02 ± 27.40	720.02 ± 70.80	876.90 ± 60.14
KREA (µmol/L)	Before	20.00 ± 5.00	20.60 ± 4.62	18.60 ± 4.22	20.20 ± 4.76
	After	33.20 ± 3.42	35.20 ± 2.39	31.60 ± 2.51	35.20 ± 1.79
GLU (mmol/L)	Before	7.32 ± 0.39	7.64 ± 0.73	8.04 ± 0.62	8.24 ± 0.95
	After	8.16 ± 0.59	7.47 ± 0.09	7.50 ± 0.70	6.66 ± 0.67*
UREA (mmol/L)	Before	6.12 ± 0.73	6.53 ± 1.22	5.52 ± 1.00	6.96 ± 1.15
	After	6.12 ± 0.72	6.68 ± 0.67	7.10 ± 1.00	7.42 ± 0.51

Data are mean ± standard deviation. * = statistical significance at $p \leq 0.05$.

kidneys were still in the normal range. Nevertheless, observations revealed an increased level of both creatinine and urea as a result of hydration, diet, and/or protein catabolism.

According to Khan and Alden (2002), pre- or post-renal mechanisms can result in an increased level of creatinine and urea. A measurement of glucose level indicated the overall rate of metabolism in rats. The amount of glucose was different based on intestinal absorbance, hepatic toxicity, and tissues' glucose intake (Smith *et al.,* 2002). The amount of glucose in the present study significantly decreased ($p < 0.05$) for male (before 8.24 ± 0.26 mg/kg; and after 6.16 ± 0.46 mg/kg) and female (before 8.24 ± 0.95 mg/kg; and after 6.66 ± 0.67 mg/kg) rats in the dose group of 5000 mg/kg in comparison to the control group. This might have resulted from low food consumption, which had eventually brought about a loss of total body fat. Next, skeletal and muscle cell damages were evaluated based on the measurement of creatinine kinase (CK) enzymes' serum levels. Based on Table 3, the levels of CK in male and female rats were within the normal range despite an increase in post-treatment. Therefore, the administration of tea extract had no damages to the skeleton and heart.

Conclusion

Hot water extraction of waste tea leaves from Sabah exhibited the possibility of cytotoxicity against the proliferation of human ovarian cancer-derived cell. Findings also revealed that ethanol extract of black tea leaves showed strong cytotoxic activity against the proliferation of human ovarian cancer-derived cell. Hence, it is necessary to further investigate the potential characteristics of tea as a cancer-prevalent. This is because the rats that were administered

with a high dose of tea might suffered from slight liver damage as a result of the toxicity. Despite the death of two female rats after 28 d of the experiment, an overall result proved that male rats were highly affected in comparison to female rats. Male rats that were administered with 5000 mg/kg of green tea extract exhibited decreased testes weight and increased liver weight. As a result, a significant increase in the amount of ALT was observed in the serum of treated male rats in the 1250 and 5000 mg/kg groups of doses, respectively. Nonetheless, the inconsistent pattern between ALT and ALP suggested a minor mechanical trauma in the liver as a result of high doses' treatment groups.

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