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Research Article

Cytotoxic Evaluation of *Eurycoma longifolia* Jack Root Extract on Chromosome Aberrations in Human Lymphocytes *In vitro*

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ABSTRACT

This study aimed to investigate chromosomal aberrations of *Eurycoma longifolia* Jack (*EL*) root extract in human lymphocytes *in vitro*. Human whole blood was cultured in medium solution that treated with distilled water, 20% DMSO, extract of *EL* roots at the concentration of 2.5, 5, 10, 20, 40, 80 µg/mL (extracted with distilled water and ethanol), and nontreated (blank: only culture medium and whole blood). All experiments were cultured for 72 hours in the 37°C incubator .The effects of *EL* roots extract on cytotoxicity were compared with the control groups including the blank, distilled water, and 20% DMSO. This study found that *EL* root extract significantly decreased metaphase cell number and increased chromosome aberrations dose dependent manner ($p<0.01$). The 7 types of chromosome aberration that were observed consisted of dicentric chromosome, single chromatid breaks, isochromatid break, isochromatid gap, single chromatid gap, fragmentation, and deletion. The dicentric chromosome was the most common chromosomal aberrations type that was treated with *EL* root extract both distilled water and ethanol. Moreover, the ethanolic extract of *EL* root was more effective to stimulate chromosome aberrations compared to the water extract of *EL* root (the deletion and fragmentation were not found in the water extract of *EL* root). This study demonstrated that the phytochemicals of *EL* root extract had cytotoxicity effect (decreased metaphase cells and increase cells death) and genotoxic effect (increased chromosomal aberrations. The use of *EL* root crude extract with distilled water is therefore safer for cells. However, when *EL* is used at high levels, it may lead to the inhibition of cell division process and cause side effects (toxicity). *EL* extracts consist of various phytochemicals with different properties and dosages, thus more studies should be conducted on the effect of those substances on cytotoxicity, especially their effects on genotoxicity humans.

INTRODUCTION

Amongst the tropical herbal plants in Southeast Asia, *Eurycoma longifolia* Jack

(*EL*) is a well-known and widely used medicinal herb. It is a native flowering and a long life of the family Simaroubaceae. There are different names for each area, such as Pasak Bumi or Bedara Pahit (Indonesia), Cay ba binh (Vietnam), Tongkat Ali, Ali's Umbrella or Malaysia ginseng (Malaysia), tho nan (Laotian), and Ian-don (Thailand) (Rehman et al. 2016). It is an evergreen, slender, tall, slow-growing shrub plant with maximum height of 15-18 m and commonly found in tropical forests. It is also popular as medicinal plant. The local people claim that the tea made from this plant can improve sexual abilities and virility (Kuo et al. 2003). It has now become a protected plant species to avoid extinction due to high demand for herbal medicines and health supplements production (Kuo et al. 2003). The *EL* root is commercially available in powdered form, formulated crude extract, capsule, tablet and is commonly used as food additives in beverages such as coffee and tea in healthy food markets in Southeast Asia. The well-known commercial trademarks of *EL* in markets are Maxidus®, LIbidus®, Passion Rx®, Tribulus®, and LJ100® (Bhat & Karim 2010). Among consumers, *EL* in the capsules with raw crude root powder or standardized *EL* extract is the most common form-of using. This standardized *EL* extract includes the processes of extracting the active ingredients, adjusting the preparation to a defining content of a constituent, and concentrating it to a standard level (Mohd et al. 2012) There is a high demand of *EL* production for herbal medicines and supplement with more than 54,000 kg per year for commercial product development and 200 *EL* root products registered with the National Phamaceutical Control Bureau of Malaysia (NPCB) (Rehman et al. 2016).

EL is considered as one of the King of herb. Its popularity is due to high amount of various bioactive compounds. The phytochemicals of *EL* root extracted and isolated by different solvents consisted of eurycomalactone, eurycomanol, triterpenes-type tirucallane, canthine-6-one alkaloid, 9-hydroxycanthine-6-one, 14,15 *β*-hydroxyklaineanone, phenolic components, eurycomanone, quanissoids, 5-hydroxymethyl-9-methoxycanthin-6-one, 1-hydroxy-9-methoxycanthin-6-one, *n*-pentyl *β*-carboline-1-propionate, and tannins (Choo & Chan 2002; Kuo et al. 2004). It has been reported since the 1980s that they were widely applied for the treatment of chronic diseases and wellbeing promotion. Because of its distinctive medicinal value, *EL* root extracts have been intensively investigated for pharmacological purposes. Several studies have performed both *in vitro* and *in vivo* focusing on the aphrodisiac, anticancer, antimalarial, antiosteoporosis, and antidiabetic properties using different assays (Segaran et al. 2021). It is also used as an anti-stress (decrease cortisol) (Talbott et al. 2013), anti-microbial (Khanam et al. 2015), appetite stimulant, health supplement (Rehman et al. 2016), immunomodulation to improve immunity (George et al. 2016), and anti-inflammatory (Han et al. 2016). The *EL* root extract was verified increasing the sexual performance in male rats. It also developed prostate and seminal vesicle's growth (Ang et al. 2000; Ang & Cheang 2001). In 2019, Hien et al. studied the effects of alkaloids from EL roots extract and found significant anti-inflammatory effect *in*

vitro and *in vivo*. They suggested that alkaloid exhibits the anti-inflammatory activity via suppression of pro-inflammatory mediators (nitric oxide (NO), inducible nitric *oxide* (iNOS), cyclooxygenase 2 (COX-2). These results demonstrated that *EL* dose-dependently protected mice from lipopolysaccharide (LPS-induced mortality) and inhibited LPS-induced NO production (decreasing NO) as well as the protein iNOS and COX-2 expressions. Therefore, the impacts of *EL* root extract are interesting due to its ability to improve immune system and to prevent diseases in humans. However, most importantly, *EL* is highly recommended to use with precaution due to its toxicity and side effects if used in excessive doses.

Studies in *EL* safety have shown that the composition of *EL* extracted with n-butanol, ethanol, and water is different. The *EL* extract with water is considered the safest among other, as its LD_{50} is comparatively higher (>3000 mg/Kg) than ethanol and n-butanol extracts (most toxic). High doses of *EL* (Tongkat Ai Plus®) (1000 mg/Kg) reduced the percentage of pregnancy rates in rats (Li et al. 2013; Aida et al. 2016). The *EL* extract showed an acute toxicity in mice. Alcoholic extract of *EL* has oral lethal dose 50 (LD_{50}) between 1500-2000 mg/kg, whereas the oral LD_{50} of the water extract of *EL* was more than 3000 mg/kg (Rehman et al. 2016). The *EL* root extract was indicated to cause hepatotoxicity in subacute toxicity via daily oral gavages for 28 days, in male rats (Shuid et al. 2011). Toxicity levels and safe doses of a major compound of *EL* eurycomanone) had been *studied* in catfish (*Clarias magur*). The 96 h LD_{50} of eurycomanone was found to be 0.3917 µg/g while the subacute toxicity level and safe dosage of eurycomanone were 0.274 µg/g (70% LD_{50}) and 0.137 µg/g (35% LD_{50}). Furthermore, there had been a significant in micronuclei, DNA damage, and biochemical parameters in the subacute toxicity groups compared to the safe dosage (35% LD_{50}) and control groups. The transcript levels of apoptotic genes were significantly higher in the subacute toxicity groups than those in the optimal dosage and control groups. According to this study, it is concluded that cytotoxic effect of eurycomanone or major compound of *EL* extract depends on level of an administration and extracting solvents (Bhat et al. 2017). Chromosomal aberration assay is a sensitive and reliable test for genotoxic experiments *in vitro*. Nonetheless, chromosome aberrations could rise from secondary mechanisms involved in cytotoxicity. Therefore, these compounds that did not react with DNA were not genetically toxic in vivo, and some positive results *in vitro* aberration assay did not exhibit cytotoxic in human (Galloway 2000). In the past, the *EL* cytotoxic effect was investigated as anti-proliferative, mutagenic effect, and chromosome aberration in animals, virus, bacteria, normal cell, as well as cancer cell. In addition to that, it is also applied as antimicrobials, anti-parasite, anticancer, and antimalaria in humans. The various *concentrations* of *EL* powdered root in the range of 1.25-5.0 mg/mL significantly affected on the cell viability (Li et al. 2013). *EL* was revealed to contain mutagenic and genotoxic substance (alkaloids β-carboline), some components of *EL* could be carry mutagenic effects in low concentration

(Razak & Aidoo 2011). Cytotoxicity effect of *EL* is greatly crucial for further studies in terms of its pharmacological activities. In the meantime, there is no study reported in human cells. This study aimed to investigate further on the cytotoxicity effect and the safety of *EL* root extracts with distilled water and ethanol. Typically, *EL* roots are boiled or pickled in liquor and were previously used as a traditional herbal remedy.

MATERIALS AND METHODS

EL root sample and extractions

EL root was washed in water for 3-4 times and cut to thin sheet, then it was sun dried for 1-2 days. After that, *EL* root was incubated at 60 °C for 4 hours. Dry root of *EL* was grinded to 200 grade mesh and extracted using a ratio of 1 part of *EL* powder per 10 parts of solvent (distilled water or ethanol). In ethanol extraction method, the mixture of *EL* roots was agitated in a shaking water bath at 30 °C for 2 hours, while the sample from distilled water extraction method was shaken at 60 °C for 6 hours. *EL* extract from two solvents were filtered and then dried by vacuum evaporator for ethanol extraction of *EL* root and dried by freeze dehydration for distilled water extraction of *EL* root. All *EL* extracts were stored at 4 °C for further experiments.

Experimental design

In treated groups, the water crude extract of *EL* roots was dissolved by distilled water while 20% DMSO was used for dissolving ethanol crude extract of *EL* roots. The final concentrations of both *EL* root extracts were 2.5, 5, 10, 20, 40, and 80 µg/mL. In a control group, whole blood cells were culture in a combination of cell culture medium (blank), distilled water, and 20% DMSO respectively without an addition of *EL* extract whilst in treatment groups, cell culture were treated with *EL* extracted with distilled water and ethanol respectively. All experiments in the treated groups and control groups consisted of 2 replications.

Human whole blood cell culture

The *EL* herbal is commonly used especially among middle age men, but it has side effects and can be dangerous for pregnant and lactating women (Turck et al. 2021). In this study, blood sample was collected (15 mL) from a male volunteer at the aged of 49 years old by the permission of the Srinakarin hospital, the aseptic technique was used during the processes of the blood collection. This study was performed according to the international, national, and institutional rules considering human experiments. The clinical studies received the permission from an ethical committee, Khon Kaen University, Faculty of Science (ID U1-04498-2559). The sample was kept on ice in 5 mL vacuum test tubes coated with heparin. Each T-25 flask for each condition comprised 0.5 mL of whole blood that was collected in 8 mL RPMI 1640 medium. In treatment groups, the sample was supplemented with 100 µL of each concentration of *EL* root extract, whereas the control groups were

filled with distilled water and 20% DMSO (blank did not added with any substance) and incubated at 37 °C with flows of 5% CO_2 for 72 hours (Freshney 2005). After incubation, colchicine was introduced and mixed for 30 minutes before a further incubation. After colchicine incubation, the blood mixture was centrifuged 1.200 rpm at room temperature for 10 minutes. After that, the supernatant was discarded by sucking it off carefully with a glass pipette. The cells were treated with hypotonic solution (0.075 M KCl) and kept at room temperature for 30 minutes. They were centrifuged and the supernatant was discarded. Fresh cool Canoy's fixative was used to fix the cells by gradually added up to 8 mL (Sangpakdee 2016). Later, to make the supernatant clear, the fixation would be repeatedly conducted (twice). The cells were finally resuspension in 1 mL fixative, cells suspensions were dripped with 2 drops (20 µL) by micropipette on a clean and cooled glass slide and followed by air drying. Finally, chromosomes were stained using the Giemsa staining. The dried slide was stained with 20% Giemsa's solution for 30 minutes (Rooney 2001).

Chromosome checking and statistical analysis

The sample in control and treated groups (6 slides/treatment) were prepared for cytotoxic evaluation. The cell counting and recording were performed in mitotic metaphase and abnormal chromosomes under the light microscope at 1,000 times magnification. Distinctly observable chromosome plates with well-spread cells were chosen and pictures were taken by digital cameras. All chromosome parameters such as metaphase index and chromosomal aberrations were analysed by one-way ANOVA as a completely randomized design (CRD) using the general linear models (GLM) procedure of SAS software (SAS® Institute, 2005). All statistical tests in this study were operated at p-value less than 0.05 ($p < 0.05$).

RESULTS AND DISCUSSION

Effect of *EL* root extract on mitotic division

Mitogen in medium culture was a stimulated factor for mitotic division of lymphocyte in human whole blood. The results of mitotic division showed that no significant difference among the control groups (blank, distilled water, and 20% DMSO) ($p > 0.05$). The mitotic division of the treated groups tended to decrease dose dependent manner and no mitotic division (no metaphase cell) was observed at the concentration of 80 µg/mL-distilled water and ethanol *EL* root extracts (Figure 1). In all treated groups with *EL* root extract, the number of metaphase cell was decreased and chromosomal aberrations were significantly increased different with the control groups ($p < 0.01$).

Effect of distilled water extract of *EL* root at the concentration of 2.5, 5, 10 and 20 µg/mL showed that the metaphase cell was significantly different with that of at the concentration of 40 µg/mL ($p < 0.01$). As well as, the result of ethanol extract of *EL* root exhibited that the number of metaphase cell and chromosomal aberration were significantly different between the concentration

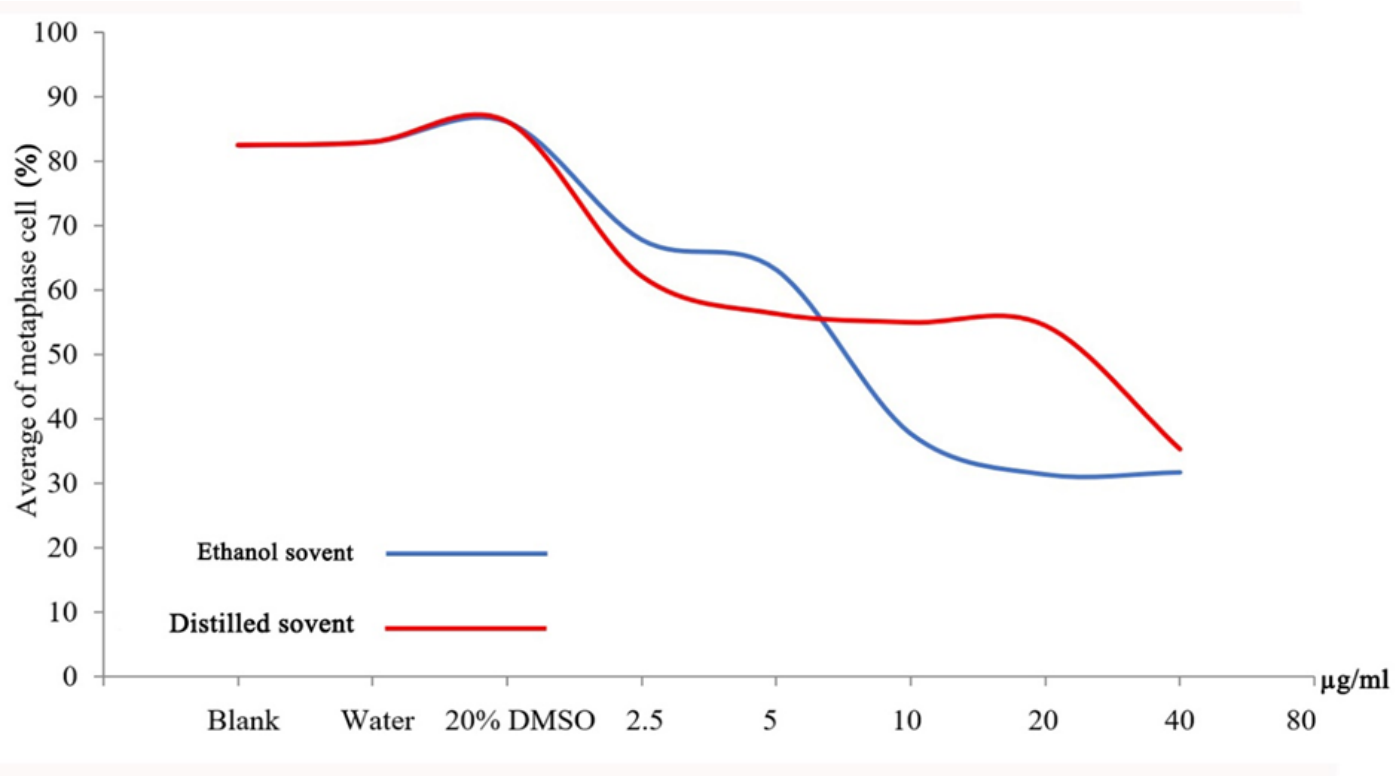

Figure 1. Decreasing of mitotic division of male human lymphocytes affected by increased concentration of distilled water and ethanol extract of *EL* roots between 2.5 - 80 µg/mL.

group of 2.5 and 5 µg/mL as well as with the concentration group of 10, 20 and 40 µg/mL (Table 1).

Effect of *EL* root extract on chromosomal aberration

Human chromosome contains 46 with 44 autosomes and 2 sex chromosomes (Figure 2). During a process of cell division, chromosomal abnormalities rarely occur thanks to the mechanism of cellular repair in a cell cycle. In this study, an extract of *EL* roots induced seven types of chromosomal aberration including

Table 1. Effects of *EL* roots extract on average and percentage of metaphase cells compared with control groups.

Control groups[1] and treated groups[2]	Water extract		Ethanol extract	
	Average of meta-phase cell (cell/20 µL)	% metaphase cell	Average of meta-phase cell (cell/20µL)	% metaphase cell
Blank	82.50 ± 1.17[a]		82.50 ± 1.17[a]	
Distilled water	83.00 ± 0.47[a]		83.00 ± 0.47[a]	
20% DMSO	86.17 ± 4.01[a]	100.00	86.17 ± 4.01[a]	100.00
2.5 µg/mL	62.17 ± 5.42[b]	72.14 ±14.85	67.83 ± 7.78[b]	78.72 ± 23.33
5 µg/mL	56.33 ± 1.89[b]	65.37 ±15.56	63.17 ± 4.01[b]	73.30 ± 12.02
10 µg/mL	55.00 ± 2.36[b]	63.83 ±7.07	37.67 ± 8.01[c]	43.71 ± 24.04
20 µg/mL	54.50 ± 2.12[b]	63.25±12.02	31.33 ± 4.72[c]	36.36 ± 14.04
40 µg/mL	35.33 ± 8.49[c]	41.00 ±1131	31.17 ± 7.78[c]	36.17 ± 23.33
80 µg/mL	0.00	0.00	0.00	0.00

Remarks: Values in the same column with different letters are significantly different ($p < 0.01$)

[1]Control groups: Blank, Distilled water, 20% DMSO

[2]Treated groups: Concentrations of *EL* root extract at 2.5, 5, 10, 20, 40, and 80 µg/mL

isochromatid break (ISCB), single chromatid gap (SCG), deletion (D), fragmentation (F), single chromatid break (SCB), isochromatid gap (ISCG), and dicentric chromosome (DC) (Figure 3). The total aberration points of all type of chromosomal aberrations in the control groups (blank, distilled water, and 20% DMSO) were 4, 8, and 5 points respectively. Whereas, the total aberration points of all type of chromosomal aberrations in the distilled water extract of *EL* roots groups at the concentration of 2.5, 5, 10, 20, and 40 μg/mL were 29, 43, 53, 55, and 53 points, respectively. Surprisingly, cell division and metaphase cells were not detected when treated with *EL* extract at the concentration of 80 μg/mL, therefore, the number of metaphase cells and chromosomal aberrations cannot be assessed. Dicentric chromosome (DC) was the most common chromosomal aberration type in human lymphocyte that treated with distilled water extract of *EL* root. The percentage of cell with chromosomal aberrations of all treated groups were significantly different compared with the control groups. The extract of *EL* roots had significantly different effects on chromosomal aberrations dose dependent manner. The extract of *EL* roots at the concentration of 40 μg/mL displayed the highest chromosomal aberration and there were significantly different compared to the *EL* roots extract at the concentration of 2.5, 5, 10, and 20 μg/mL ($p < 0.01$) (Table 2).

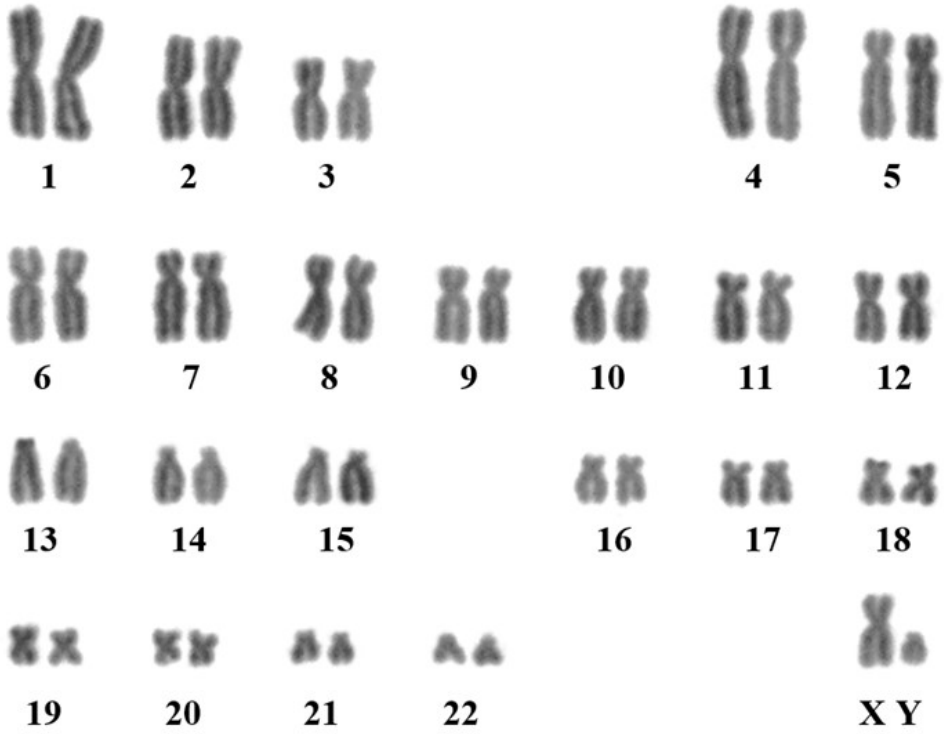

Figure 2. Human normal karyotype from normal metaphase cell of this study (control group).

When the cell treated with ethanol extract of *EL* root, the total points of chromosomal aberration types in test groups, including the control group (blank, distilled water, 20% DMSO), at the concentration of 2.5, 5, 10, 20, 40, and 80 μg/mL were 4, 9, 5, 25, 24, 33, 53, 77 and no cell division, respectively. Total 84 dicentric chromosomes (DC) which were observed were the most common ab-

erration type in ethanol extract of *EL* root groups. The number of chromosome aberration of treated groups was significantly different when compared to the control groups ($p < 0.01$). In addition, there were significantly different chromosome aberrations in various concentration groups of ethanol extract of *EL* root ($p < 0.01$) (Table 3).

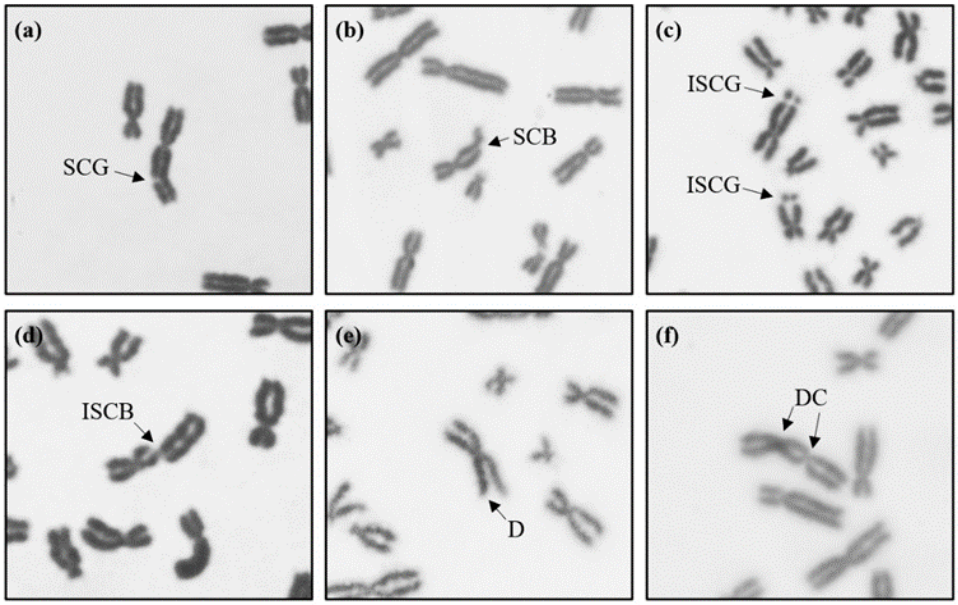

Figure 3. Six types of metaphase chromosome abnormalities in male human lymphocytes *in vitro* affected by *EL* root extract, such as single chromatid gap (SCG), single chromatid break (SCB), isochromatid gap (ISCG), isochromatid break (ISCB), deletion (D), and dicentric chromosome (DC).

Table 2. Effects of distilled water extract of *EL* roots on number and percentage of chromosomal aberrations in human lymphocytes *in vitro*.

Control group[1] and treated group[2]	Number of abnormality points							Total of abnormal points	Total of abnormal cells	The percentage of cell number with aberrations (mean ± SD)
	SCG	ISCG	SCB	ISCB	D	F	DC			
Blank	2	0	1	1	0	0	0	4	4	0.81 ± 0.01[a]
Distilled water	4	2	1	1	0	0	0	8	7	1.41 ± 0.29[a]
20% DMSO	3	0	1	1	0	0	0	5	5	0.96 ± 0.23[a]
2.5 µg/mL	6	5	4	3	0	0	11	29	29	7.89 ± 2.58[b]
5 µg/mL	8	10	3	2	0	0	20	43	41	12.16 ± 1.66[c]
10 µg/mL	16	11	1	3	0	0	22	53	51	15.44 ± 0.62[c]
20 µg/mL	14	13	5	4	0	0	19	55	52	15.96 ± 3.22[c]
40 µg/mL	19	12	4	3	0	0	15	53	49	23.22 ± 0.91[d]
80 µg/mL	0	0	0	0	0	0	0	0	0	0.00
Total	72	53	20	18	0	0	87	250	238	

Remarks: SCG= single chromatid gap, ISCG= isochromatid gap, SCB= single chromatid break, ISCB=isochromatid break, D= deletion, F= fragmentation and DC= dicentric chromosome.

Values in the same column with different letters are significant different ($p < 0.01$)

[1]Control groups: Blank, Distilled water, 20% DMSO,

[2]Treated groups: *EL* root extract at the concentration of 2.5, 5, 10, 20, 40, and 80 µg/mL

Table 3. Effects of the ethanol extract of *EL* root on number and percentage of chromosomal aberrations in human lymphocy.

Control group1 and treated group2	Number of abnormality points							Total of abnormal points	Total of abnormal cells	The percentage of cell number with aberrations (mean ± SD)
	SCG	ISCG	SCB	ISCB	D	F	DC			
Blank	2	0	1	1	0	0	0	4	4	0.81 ± 0.01[a]
Distilled water	4	2	2	0	1	0	0	9	8	1.41 ± 0.29[a]
20% DMSO	3	0	1	1	0	0	0	5	5	0.96 ± 0.23[a]
2.5 µg/mL	9	4	2	2	1	0	7	25	25	6.20 ± 1.06[b]
5 µg/mL	4	2	1	1	3	2	11	24	23	6.21 ± 4.05[b]
10 µg/mL	7	6	4	1	2	0	13	33	33	14.60 ± 0.03[c]
20 µg/mL	14	8	6	2	2	2	19	53	49	26.76 ± 9.29[d]
40 µg/mL	15	15	4	5	3	1	34	77	70	36.30 ± 9.09[e]
80 µg/mL	0	0	0	0	0	0	0	0	0	0.00
Total	58	37	21	13	12	5	84	230	217	

Remarks: SCG= single chromatid gap, ISCG= isochromatid gap, SCB= single chromatid break, ISCB= isochromatid break, D= deletion, F= fragmentation and DC= dicentric chromosome

Values in the same column with different letters are significant different ($p < 0.01$)

[1]Control groups: Blank, Distilled water, 20% DMSO,

[2]Treated groups: *EL* root extract at the concentration of 2.5, 5, 10, 20, 40, and 80 µg/mL

This study showed that various compounds of ethanol extract of *El* root carried higher level of cytotoxicity than those distilled water extracted. The ethanol extract of *EL* roots and distilled water presented cytotoxic activities, the half maximum inhibitory concentration (IC_{50}) on cell division were between the range of 5-10 and 20-40 µg/mL, respectively (Table 1). Some of these results differ from previous studies with cells microorganisms. Kavitha et al. (2010), showed that all fraction from *EL* root possessed a moderate cytotoxicity activity against Vero cell, the 50% cytotoxicity concentration (CC_{50}) value was 5-23.50 µg/mL. In the study of Ming et al. (2014), it was indicated that aqueous extract of *EL* roots at dose <5 µg/plate did not show any cytotoxic effect on *Salmonella* spp. *in vitro*. The results showed that the ethanol extract of *EL* roots was more effective to chromosomal aberrations than the distilled water extract of *EL* roots. The effect of *EL* toxicity on the mitotic index (metaphase cell number) was statistically significant ($p<0.01$) in both groups of the ethanol extract of *EL* roots and distilled water. Hence, the decreases of cells during metaphase in the groups treated with *EL* root extracts may be strongly suggested that components in *EL* extracts act as cytotoxic agents. In accordance with *in vitro* studies, the compounds found in the extracts are also associated with cytotoxic effects in humans. Furthermore, the outcome of chromosomal aberration in each test group which affected from *El* roots extracted with distilled water exhibited the significant difference (Table 2) which was in an agreement with the results from the group treated with the ethanol extracts (Table 3). Significant decrease of cell division at higher concentrations (40-80 µg/mL) is due to the suppression of DNA synthesis at S phase of cell cycle as well as-RNA and DNA biosynthesis were impaired and thus formation of nucleic acid and protein was de-

clined (Qari & El-Assouli 2019). It is possible that compounds at high concentrations in crude extracts lead to irreversible DNA damage in lymphocytes (genotoxic effect) and cell death (cytotoxic effect) by inhibiting mitotic event (Askin & Asanturk 2018).

Dicentric chromosome was the most common aberration type that treated with distilled water and ethanol extract of *EL* root groups. In the distilled water extracted groups were discovered five aberration types such as SCG, ISCG, SCB, ISCB, and DC while in the ethanol extract groups were found seven aberration types (SCG, ISCG, SCB, ISCB, D, F, DC) of abnormal chromosome were determined. Deletion and fragmentation were found only in cells treated with ethanolic extract-of *EL* roots which completely cause the breakage chromosome. The completely breakage chromosome was the non-appearing of a little fragment on main centric chromosome (deleted and fragment), but the non-completely breakage chromosome was the appearing of a little fragment on main centric chromosome (gab, break, and dicentric). In the distilled water extract of *EL* root groups cellular samples were only aberration in the non-completely breakage chromosome, but in the ethanolic extract groups, both completely and non-completely breakage chromosomes were observed in the samples. These results may indicate that the presence of one or more components of *EL* root extract is able to break the DNA strands in DNA in a certain way, that may lead to chromatin gap due to deletion or absence of one or more nuclei and couldn't be repaired by the repair mechanism (Qari & El-Assouli 2019). The result from the study in the particle of *EL* root isolated suggested that several compounds such as eurycomanone, eurycomalactone and alkaloids β-carboline may cause acute cytotoxic, genotoxic and mutation. Several investigations reported that *EL* extracts induce side effects on human lymphocyte as well as in animals such as mice, rats, and catfish (Razak & Aidoo et al. 2011; Rehman et al. 2016; Bhat et al. 2017). When consumed by pregnant women, substances in *EL* have direct effects on foetal development by inducing chromosomal abnormalities due to its cytotoxicity. It is also known as a major cause pregnancy loss in humans with more than 90% of early abortions were contributed by foetal aneuploidy (Low et al. 2013).

Currently, prolonged consumption of *EL* root extract has associated with a range of side effects, including sleep apnea, facial flushing, pressure in the testicles, and hyper-aggressiveness, but there is no scientific evidence to support the mechanism of action. In clinical practice, specific dosages of *EL* (water extract) in humans have been suggested and supplementation of the extract for 2-8 weeks at doses of 100-600 mg/day had a pharmacological effect in men (Tambi 2005). Besides that, Bhat & Karim (2010) recommend a safe daily dose of *EL* root extract between 270–350 mg/kg with no cytotoxic effects. Shuid et al. (2011) suggested that oral administration of *EL* extracted with water and ethanol at 300 and 200 mg/Kg/day, respectively did show any sign of toxicity. It should be noted, that we do not recommend to using the *EL* root extracted with ethanol (pickled in liquor) regarding the safety issue (cytotoxicity). An intake of *EL* roots powder including capsules, bolus,

coffee, and tea (water extraction) is better way to prevent cytotoxic effect in consumers. Most importantly, *EL* extract should be used at appropriate level and thereby bioactive components can be excreted from the body without being accumulated in a large amount that may induce health problems in long term consumption.

CONCLUSION

This study revealed that the herbal extraction method had an effect on various types and quantities of phytochemicals in the crude extract. The phytochemicals of *EL* extracts one or several compounds have a cytotoxic effect and affected the process of cell division or mitotic cell index. The ethanol extract of *EL* roots showed more severe cytotoxic effects than those distilled water extracts. High concentrations of *EL* root extracts contributed an effect (cytotoxicity) on a cell reduction during metaphase and increased chromosomal aberrations (genotoxicity) in human lymphocytes in vitro. Hence, the decrease of the MI values obtained in the blood lymphocytes indicated that *EL* root extract may be suggested as cytotoxic agents. Aqueous extracts are safer than ethanol extracts and the higher concentrations, the susceptible to cytotoxic effect it is. This information is valuable to consumers for a safe selection on *EL* root extract as well as for a production of *EL* extract as medicines and nutritional supplements.

AUTHORS CONTRIBUTION

SC, IP designed the study. SC, SP performed laboratory work. SC, IP, SP analyzed the data. SP, CS, AT wrote the manuscript. All authors read and approved the final version of the manuscript.

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CONFLICT OF INTEREST

The authors declared that there is no conflict of interest.

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