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SAFETY EVALUATION OF THE ETHYL ACETATE EXTRACT ON IRRADIATED TEA PARASITE: ACUTE TOXICITY STUDY ON MICE

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ABSTRACT

SAFETY EVALUATION OF THE ETHYL ACETATE EXTRACT ON IRRADIATED TEA PARASITE: ACUTE TOXICITY STUDY ON MICE. Many studies of the pharmacological efficacy of tea parasite and the use of ionizing radiation for decontamination of microbes and extending shelf life have been reported, but there is no information on its safety, such as the acute toxicity. In this study, the acute toxicity of two ethyl acetate extracts from unirradiated and irradiated (irradiation dose of 10 kGy) tea parasites *Scurrula atropurpurea* on *Swiss Webster* mice have been examined. The observation was done after the treatment of a single oral dose of ethyl acetate extract in various dose groups, i.e.: control (0 g/kg of mice body weight), D1 (0.625 g/kg), D2 (1.25 g/kg), D3 (2.5 g/kg) D4 (5 g/kg), D5 (10 g/kg) by observing the effect on behavioral response (pharmacological profile), the body weight gains and mortality until the day 14^{th}. At the last day, the observation of vital organs has also been done. The result showed that no acute toxicity was found in mice treated with a single oral dose of ethyl acetate extract from unirradiated tea parasite and irradiated tea parasite at the dose of 10 kGy. At the dose up to 10 g/kg (equivalent to 77.6 g of extract which administered to human), the normal body weight gains were observed in mice of all dose groups, no mice deaths in any of the dose groups, and no significant change ($p > 0.05$) in organ weights relative to the body weight i.e.: liver, spleen, kidneys, lung, heart, testes and seminal vesicle (for male), and ovaries and uterus (for female). The approximate lethal doses for male and female mice were determined to be higher than 10 g/kg of mice body weight. It is suggested that the treatment of ethyl acetate extract from unirradiated and irradiated tea parasites until dose up to 10 g/kg of mice body weight was still safe.

Keywords: acute toxicity, tea parasite, *Scurrula atropurpurea,* ethyl acetate extract

ABSTRAK

EVALUASI KEAMANAN EKSTRAK ETIL ASETAT DARI BENALU TEH YANG DIIRADIASI: STUDI TOKSISITAS AKUT PADA MENCIT. Beberapa studi khasiat farmakologis ekstrak benalu teh dan penggunaan radiasi pengion untuk dekontaminasi mikroba dan memperpanjang masa simpan telah dilaporkan, tetapi tidak ada informasi tentang keamanan, misalnya toksisitas akut. Dalam studi ini, toksisitas akut dua buah ekstrak etil asetat dari sampel benalu teh *Scurrula atropurpurea* yang tidak diiradiasi dan diiradiasi (dosis radiasi 10 kGy) pada mencit *Swiss Webster* telah diuji. Pengamatan dilakukan setelah pemberian ekstrak etil asetat dosis tunggal secara oral dalam berbagai kelompok dosis, yaitu: kontrol (0 g/kg berat badan mencit), D1 (0,625 g/kg), D2 (1,25 g/kg), D3 (2,5 g/kg) D4 (5 g/kg), D5 (10 g/kg) berupa respon perilaku (profil farmakologis), pertambahan berat badan dan kematian sampai hari ke 14. Pada hari terakhir, pengamatan organ-organ vital juga dilakukan. Hasil penelitian menunjukkan bahwa tidak ditemukan toksisitas akut pada mencit yang diberi dosis tunggal secara oral dengan ekstrak etil asetat dari benalu teh yang tidak diiradiasi dan diiradiasi pada dosis 10 kGy. Pada pemberian ekstrak sampai dengan dosis 10 g/kg (setara dengan 77,6 g ekstrak yang diberikan kepada manusia), pertambahan berat badan secara normal teramati pada mencit untuk semua kelompok dosis, tidak ada kematian mencit dalam semua kelompok dosis, dan tidak ada perubahan signifikan ($p > 0,05$) berat organ relatif terhadap berat badan pada semua organ, yaitu: hati, limpa, ginjal, paru-paru, jantung, testis dan vesikal seminalis (untuk jantan), dan ovarium dan uterus (untuk betina). Prakiraan dosis letal untuk mencit jantan dan betina lebih tinggi dari 10 g/kg berat badan mencit. Diduga bahwa pemberian ekstrak etil asetat dari

benalu teh yang tidak diiradiasi dan diiradiasi sampai dosis 10 g/kg berat badan mencit masih aman.

Kata kunci: toksisitas akut, benalu teh, *Scurrula atropurpurea,* ekstrak etil asetat

1. INTRODUCTION

In the recent years, the use of herbal medicine for complementary treatments of some diseases has been popular. Some herbal medicine industries have used various medicinal plants, including tea parasite (*Scurrula atropurpurea* Bl. Dans.) as a raw material of herbal medicine for cancer treatment. Tea parasite have been known to contain many active compounds as anti-cancer potential, and among the compounds of octadeca-8,10,12-tryinoic acid, (*Z*)-octadeca-12-ene-8-10-dienoic acid, and (-)epigallocatechin-3-*O*-gallate exhibit more potent inhibitory effect on cancer cell invasion MM1 *in vitro* (1).

The use of herbal medicine for treatment of some deseases is a good choice, since it is cheaper than synthetic drugs. In addition, synthetic drugs are believed to cause various side effects. Nevertheless, dry herbs are easily damaged during storage due to microbial contamination, cause the reduction of the efficacy and quality of herbal medicines. To overcome this problem, ionizing radiation can be used. Codex Alimentarius Commission states that ionizing radiation has been used to decontaminate the pathogenic microbes and to extend the shelf life of herbs, foods, and spices with the maximum absorbed dose should not exceed 10 kGy, except when necessary to achieve legitimate technological purposes (2). In Indonesia the use of ionizing radiation for sterilization of products has been regulated by the Minister of Health No. 701/MENKES/PER/VIII/2009 (3). Irradiation dose up to 10 kGy can reduce the number of bacteria on *Phaleria macrocarpa* Scheff. Boerl. dried powder from 10^{10} cfu/g to 10^{5} cfu/g (4), this value is qualified according to The National Agency of Drug and Food Control (NADFC) or *Badan Pengawas Obat dan Makanan* (BPOM) (5). Gamma irradiation technique as one of ionizing radiation has several advantages including high penetrating energy, can be applied at room temperature, leaving no residue, environmental friendly, and can be packed in the end products (2,5).

In spite of gamma irradiation has been used by some herbal medicine industries, but the effect of gamma irradiation on the efficacies has not been studied yet. In the previous study, gamma irradiation with doses up to 7.5 kGy on the bark of *Phaleria macrocarpa* Scheff. Boerl. was used for decontamination of pathogenic microbes and to extend the shelf life without affecting the cytotoxic activity on L1210 leukemia cells (4). Gamma irradiation at doses of ≥ 5 kGy on tea parasite herb (*Scurrula atropurpurea* Bl. Dans.) could inhibit the growth of molds and yeast. Gamma irradiation at doses of ≤ 10 kGy in addition was able to reduce microbial contamination

of tea parasite herb without change the cytotoxic activity in fractions 2 which is the most active fraction in ethyl acetate (EtOAc) extract, and the chromatogram profiles of components in fraction 2. Therefore, gamma irradiation at the dose of 10 kGy is the maximum dose appropriate for tea parasite herb preservation because it does not change the cytotoxic activity as anticancer agent (6). However, there is no information on its safety, such as acute toxicity.

In this report, the EtOAc extract from unirradiated and irradiated tea parasite samples at the dose of 10 kGy were examined for the acute toxicity in mice. The examination of acute toxicity using *Swiss Webster* mice was done to confirm that EtOAc extract from irradiated tea parasite at the dose of 10 kGy does not exhibit toxic effect to consumers/patients. Acute toxicity is the toxicity which occurs in short time after orally single dose treatment with test samples. All of test samples which will be applied to patient/consumer should be passed from acute toxicity test on animals in order to find the figure of risk-benefit of the test samples use (7-10).

The aim of this study is to examine the acute toxicity of the EtOAc extract from irradiated tea parasite at the dose of 10 kGy. In addition, trough acute toxicity test, the figure of LD_{50} (lethal dose 50) and the dose levels for the next test such as sub-cronic and cronic toxicity tests can be determined. The LD_{50} value is the dose which exhibit 50% death of tested animals. The extracts can be stated "toxic" if it causes the animals tested died after treated with the extracts at the dose of 5 g/kg of animal body weight (BW). So, in this study, the concentration used is between 0.625 to 10 g/kg body weight of mice as test animals.

2. EXPERIMENTAL METHODS

2.1. Materials

Fresh tea parasite *Scurrula atropurpurea* (Bl.) Dans. was collected from Gunung Mas Plantation, Bogor in January 2008. The authentication of the plant materials was carried out at the Herbarium Bogoriensis - Research Center for Biology, Indonesian Institute of Sciences, Cibinong. The voucher specimens have been kept for identifying the plant species and voucher specimens of these plants were deposited. Male and female *Swiss Webster* mice of 2 months old were purchased from Life Sciences Laboratory, Bandung Institute of Technology. Another chemicals used were acetone, ethyl acetate, carboxy methyll cellulose-sodium salt (CMC-Na).

2.2. Equipments

Equipment used in this research were *Karet Alam* Irradiator (IRKA) PATIR – BATAN which is gamma irradiator from cobalt-60 source with the activity was 140 kCi at January 14, 2008, rotary evaporator, mortar and stamper, oral sonde, balance for mice, surgery apparatus, and other apparatus for acute toxicity test.

2.3. Samples irradiation

Dried tea parasite herbs were powdered, weighed (1,700 g), packed in polyethylene bag and sealed with sealer machine. They were prepared into 2 bags, one bag was irradiated at doses of 10 kGy

with dose rate of 10 kGy/h at *Karet Alam* Irradiator, and the other was unirradiated.

2.4. Extract preparation

The irradiated and unirradiated samples were macerated in 70% acetone for 48 hours and the filtrates were filtered. The residue was macerated again for three times, then the filtrates were combined and evaporated with rotary evaporator to remove acetone. The obtained extracts were then partitioned into EtOAc-water (1:1). The EtOAc-soluble portion was concentrated and dried using oven desiccator *in vacuo*, then weighed.

2.5. Acute toxicity test

The acute toxicity test was done according to the OECD guideline for testing of chemicals (8,9) and the traditional drug regulation (10). Mice were acclimatized for 1 week, only the healthy mice were used in the experiment. The examined EtOAc extract obtained from irradiated and unirradiated tea parasites with six various doses i.e. 0 g/kg BW of mice (control, C), 0.625 g/kg BW (D1), 1.25 g/kg BW (D2), 2.5 g/kg BW (D3), 5 g/kg BW (D4), and 10 g/kg BW (D5) were suspended in 0.5 % of CMC-Na (sodium carboxymethyl cellulose) solution, then orally treated in single dose per group. Every group contained 10 male and 10 female mice. The observation were done towards the appeared effects for two minutes every hour within first four hours interval, such as the effect on central nervous system, autonomous nervous system, reflex, breath rhythm, change in secretion, skin and mucose condition, body posture, heart pulse rate, and other responses which generally observed on acute toxicity test.

The gain of body weight and mice death were observed continuously every day until the day fourteenth after treated with EtOAc extract. The obtained mice death was immediately dissected to determine death causative. At the day fourteenth, all mice were killed then the organs were weighed and calculated for relative organ weight toward body weight. The organs of male mice observed were liver, spleen, lung, kidneys, heart, testes and seminal vesicle, while for female mice were liver, spleen, lung, kidneys, heart, ovaries and uterus. The body weight gained was also observed. The signification of obtained data was statistically calculated using t-test at $p < 0.05$ (11).

3. RESULTS AND DISCUSSION

Extraction of 1,700 g of irradiated and unirradiated dried tea parasite herbs gave EtOAc extract of 122 g (7.2%), respectively. The extracts then were used for acute toxicity test.

3.1. Profile of pharmacological activity

Pharmacological activity profiles of mice after treated with a single oral dose of EtOAc extract from unirradiated tea parasite, showed that male and female mice of all tested and control groups generally did not change in pharmacological activity, except some activities (Table 1).

In male mice, grooming, defecation, and urination occurred in all of tested groups except in D3 group, urination did not occur,

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while decrease in motoric activity occurred in D3, D4, D5 groups each on one mouse (10%), flexion and hafner was occurred in D4 group on one mouse (10%), in D2 and D3 groups one mouse (10%) was being lost hanging and retablishment abilities, and in D4 and D5 groups one until two mice (10-20%) increased respiratory rate, respectively. In female mice, all tested and control groups generally changed in motoric activity, grooming, defecation, and urination. Nevertheless, treatment of EtOAc extract at the dose up to 10 g/kg BW did not cause catalepsy, vasodilatation, motion, body position disorder, lacrimation, and did not disturb the reflex and other effects.

Pharmacologic activity profile of mice after treated with a single oral dose of EtOAc extract from irradiated tea parasite (Table 2) showed that generally male and female mice of all tested groups and control group changed the motoric activity (1-3 mice), grooming (1-3 mice), defecation (3-7 mice), and urination (1-3 mice). At the dose up to 10 g/kg BW, the EtOAc extract from irradiated tea parasite did not did not cause catalepsy, vasodilatation, motion, body position disorder, lacrimation, and did not disturb the reflex and other effects.

Table 1. Pharmacological activity profiles of male and female mice during 4 hours observation after treated with a single oral dose of EtOAc extract from unirradiated tea parasite

Pharmacological activity profiles	**The number of male and female mice gave response during 4 hours observation after treated with EtOAc extract in various doses**											
	Control 0 g/kg BW		**Dose 1 0.625 /kg BW**		**Dose 2 1.25 /kg BW**		**Dose 3 2.5 /kg BW**		**Dose 4 5 /kg BW**		**Dose 5 10 /kg BW**	
	M	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**	**M**	**F**
decrease in motoric activity	0	1	0	1	0	1	1	1	1	1	1	2
pilo-erection	0	0	0	0	0	0	0	0	0	0	0	0
ptoris	0	0	0	0	0	0	0	0	0	0	0	0
corneal reflex	10	10	10	10	10	10	10	10	10	10	10	10
pineal reflex	10	10	10	10	10	10	10	10	10	10	10	10
lacrimation	0	0	0	0	0	0	0	0	0	0	0	0
catalepsy	0	0	0	0	0	0	0	0	0	0	0	0
vasodilatation	0	0	0	0	0	0	0	0	0	0	0	0
lose a hanging ability	0	0	0	0	1	0	1	0	1	0	0	0
lose a retablishmant ability	0	0	0	0	1	0	1	0	0	0	0	0
flexion	0	0	0	0	0	0	0	0	1	0	0	0
hafner	0	0	0	0	0	0	0	0	1	0	0	0
grooming	2	1	1	1	1	2	2	3	6	1	1	1
tremor	0	0	0	1	0	0	0	0	0	0	0	0
vocalization	0	0	0	0	0	0	0	0	0	0	0	0
urination	6	1	1	1	2	2	0	0	2	1	1	1
defecation	9	4	2	2	2	4	2	1	1	3	2	1
abnormal heartbeat	0	0	0	0	0	0	0	0	0	0	0	0
salivation	0	0	0	0	0	0	0	0	0	0	0	0
body position disorder	0	0	0	0	0	0	0	0	0	0	0	0
convulsion	0	0	0	0	0	0	0	0	0	0	0	0
increased respiratory rate	0	0	0	0	0	0	0	0	1-2	0	1-2	0
mortality	0	0	0	0	0	0	0	0	0	0	0	0

M: Male; F: female

Table 2. Pharmacological activity profiles of male and female mice during 4 hours observation after treated with a single oral dose of EtOAc extract from irradiated tea parasite

Pharmacological activity profiles	The number of male and female mice gave response during 4 hours observation after treated with EtOAc extract in various doses											
	Control 0 g/kg BW		Dose 1 0.625 /kg BW		Dose 2 1.25 /kg BW		Dose 3 2.5 /kg BW		Dose 4 5 /kg BW		Dose 5 10 /kg BW	
	M	F	M	F	M	F	M	F	M	F	M	F
decrease in motoric activity	1	1	2	2	2	1	3	3	2	3	1	1
pilo-erection	0	0	0	0	0	0	0	0	0	0	0	0
ptoris	0	0	0	0	0	0	0	0	0	0	0	0
corneal reflex	10	10	10	10	10	10	10	10	10	10	10	10
pineal reflex	10	10	10	10	10	10	10	10	10	10	10	10
lacrimation	0	0	0	0	0	0	0	0	0	0	0	0
catalepsy	0	0	0	0	0	0	0	0	0	0	0	0
vasodilatation	0	0	0	0	0	0	0	0	0	0	0	0
lose a hanging ability	0	0	0	0	0	0	0	0	0	0	0	0
lose a retablishmant ability	0	0	0	0	0	0	0	0	0	0	0	0
flexion	0	0	0	0	0	0	0	0	0	0	0	0
hafner	10	10	10	10	10	10	10	10	10	10	10	10
grooming	2	1	1	2	1	1	1	1	2	3	3	1
tremor	0	0	0	0	0	0	0	0	0	0	0	0
vocalization	0	0	0	0	0	0	0	0	0	0	0	0
urination	1	1	2	3	1	1	3	1	1	1	3	1
defecation	5	4	5	3	7	4	7	4	5	4	7	4
abnormal heartbeat	0	0	0	0	0	0	0	0	0	0	0	0
salivation	0	0	0	0	0	0	0	0	0	0	0	0
body position disorder	0	0	0	0	0	0	0	0	0	0	0	0
convulsion	0	0	0	0	0	0	0	0	0	0	0	0
increased respiratory rate	0	0	0	0	0	0	0	0	0	0	0	0
mortality	0	0	0	0	0	0	0	0	0	0	0	0

M: Male; F: female

3.2. Changes of mice body weight

Generally, normal body weight gains were observed in males and females of all dose groups. The mice body weight slightly increased up to the day 14th in control and mice treated with EtOAc extracts from unirradiated and irradiated samples (Fig. 1 and 2).

Except control group in male mice which have been treated with EtOAc extract from unirradiated sample, the mice body weight decreased on the day fifth, but slightly increased on the day 10 and 14. No significant differences were observed between the control group and EtOAc extract treatment groups both from unirradiated tea parasite ($p < 0.05$) and irradiated tea parasite ($p > 0.05$). No abnormal gross findings were observed in any mice. It is indicated that EtOAc extract from unirradiated and irradiated tea parasite have no a significant effect to mice.

3.3. Observation of mice mortality and lethal dose 50 (LD_{50})

During the observation period until the day 14th after treated with EtOAc extract both from unirradiated and irradiated tea parasite, there were no mice death in any groups. The doses of 10 g/kg body weight of mice were equivalent to 77.6 g of extract which administered to human. The results

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showed that the EtOAc extracts both from irradiated and unirradiated tea parasite at dose up to 10 g/kg BW of mice had no effect on mortality.

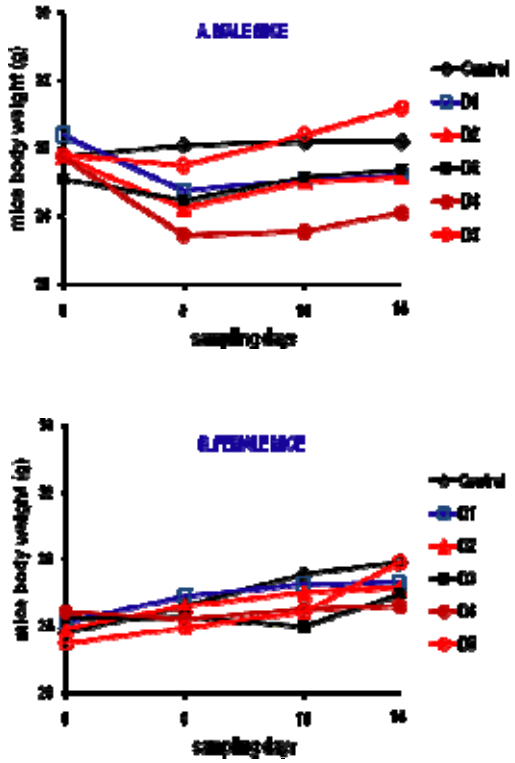

Fig 1. Mean body weight changes of male (A) and female (B) mice after treated with a single oral dose of EtOAc extract from unirradiated tea parasite at various dose level

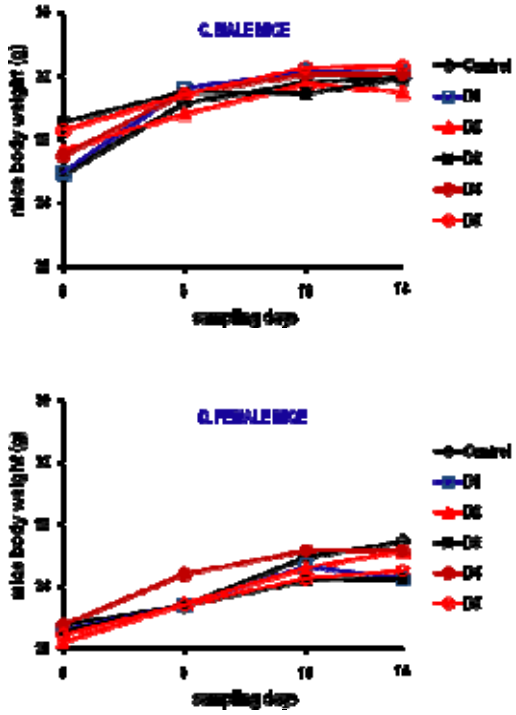

Fig 2. Mean body weight changes of male (C) and female (D) mice after treated with a single oral dose of EtOAc extract from irradiated tea parasite at various dose level

In acute toxicity test, the highest dose tested was 10 g/kg BW of mice and up to that dose no mice deaths in any of the groups both male and female mice. The results indicated that the LD_{50} of EtOAc extract both from unirradiated and irradiated (irradiation dose of 10 kGy) tea parasite were determined to be higher than 10 g/kg BW of mice.

3.4. Effects on the organ of mice

The relative organ weights (organ to body weight ratio) of male and female mice which were observed at day 14th after treated with a single oral dose of EtOAc extract from unirradiated sample can be seen in Table 3 and 4.

Visually, until the dose up to 10 mg/kg BW of mice, no significant change in relative organ weights ($p > 0.05$). Both in male and female mice (Table 3 and 4), the relative weight of liver (liver to body weight ratio), spleen, kidneys, lung, heart, testes and seminal vesicle (for male), and ovaries and uterus (for female) slightly different to control group, but not statistically different at $p > 0.05$.

Similar results of male and female mice organ profile after treated with a single

oral dose of ethyl acetate extract of tea parasite which have been irradiated at 10 kGy can be seen in Table 5 and 6. All of tested groups were statistically not different in relative organ weights i.e: liver (toward mice body weight), spleen, kidneys, lung, heart, testes and seminal vesicle (for male). However, for female, the ovaries and uterus profile was slightly different to control group, but not statistically different at $p > 0.05$.

During 14 days of observation period after treated with a single oral dose of EtOAc extract from irradiated tea parasite it was also no visible effect of extract on the eye mucosa, skin and hair of both of male and female mice. In acute toxicity test of ethyl acetate extract from tea parasite, the highest dose tested was 10 g/kg mice. This dose was slightly higher than the dose limit required to be tested. According to the guideline for testing of chemicals (7,8,10) when the dose of 5 g/kg BW does not give no significant toxic effects and no tested animals died during experiment, the use of higher dose is not necessary.

Table 3. Relative organ weight (organ to body weight ratio) of male mice which were observed at day 14th after treated with a single oral dose of EtOAc extract from unirradiated tea parasite

Test group (dose per kg BW)	Relative organ weight (organ weight to body weight ratio)						
	Liver	Spleen	Kidneys (right+left)	Lung	Heart	Testes (right+left)	Seminal vesicle
Control (0 g)	5.59±0.87	1.09±0.28	1.28±0.17	0.90±0.43	0.54±0.06	0.57±0.07	0.41±0.17
Dose 1 (0.625 g)	5.48±0.81	1.16±0.46	1.31±0.25	0.78±0.14	0.51±0.07	0.61±0.15	0.31±0.07
Dose 2 (1.25 g)	5.07±0.59	1.07±0.36	1.30±0.17	0.94±0.46	0.50±0.08	0.58±0.13	0.34±0.12
Dose 3 (2.5 g)	5.15±0.61	1.09±0.23	1.28±0.26	0.81±0.21	0.61±0.26	0.60±0.12	0.45±0.19
Dose 4 (5 g)	5.58±0.74	1.14±0.34	1.30±0.18	0.76±0.24	0.47±0.09	0.60±0.20	0.43±0.19
Dose 5 (10 g)	5.82±0.72	0.96±0.18	1.36±0.17	0.65±0.12	0.48±0.06	0.60±0.08	0.49±0.11

Table 4. Relative organ weight (organ to body weight ratio) of female mice which were observed at day 14th after treated with a single oral dose of EtOAc extract from unirradiated tea parasite

Test group (dose per kg BW)	Relative organ weight (organ weight to body weight ratio)					
	Liver	Spleen	Kidneys (right+left)	Lung	Heart	Ovaries + Uterus
Control (0 g)	4.91±0.30	0.87±0.29	0.98±0.10	0.65±0.06	0.45±0.05	0.46±0.24
Dose 1 (0.625 g)	5.07±0.69	1.06±0.22	0.99±0.11	0.73±0.12	0.50±0.09	0.56±0.23
Dose 2 (1.25 g)	5.11±0.53	0.80±0.28	1.07±0.09	0.75±0.11	0.48±0.05	0.33±0.13
Dose 3 (2.5 g)	4.75±0.63	0.70±0.28	1.06±0.11	0.71±0.10	0.47±0.06	0.45±0.33
Dose 4 (5 g)	4.98±0,86	0.70±0.37	1.07±0.11	0.74±0.15	0.46±0.04	0.41±0.27
Dose 5 (10 g)	5.32±0.60	0.86±0.22	1.03±0.05	0.73±0.10	0.49±0.04	0.46±0.17

Table 5. Relative organ weight (organ to body weight ratio) of male mice which were observed at day 14th after treated with a single oral dose of EtOAc extract from irradiated tea parasite

Test group (dose per kg BW)	Relative organ weight (organ weight to body weight ratio)						
	Liver	Spleen	Kidneys (right+left)	Lung	Heart	Testes (right+left)	Seminal vesicle
Control (0 g)	5.63±1.01	0.55±0.30	1.37±0.27	0.57±0.08	0.48±0.16	0.43±0.14	0.46±0.09
Dose 1 (0.625 g)	5.15±0.31	0.55±0.28	1.33±0.18	0.58±0.17	0.46±0.66	0.49±0.12	0.53±0.23
Dose 2 (1.25 g)	4.82±0.40	0.58±0.17	1.39±0.15	0.51±0.07	0.48±0.08	0.53±0.10	0.53±0.09
Dose 3 (2.5 g)	5.01±0.64	0.46±0.08	1.42±0.12	0.53±0.07	0.47±0.07	0.47±0.08	0.46±0.08
Dose 4 (5 g)	5.77±1.06	0.70±0.25	1.20±0.17	0.59±0.09	0.44±0.05	0.45±0.11	0.52±0.13
Dose 5 (10 g)	4.31±0.91	0.68±0.46	1.31±0.16	0.53±0.09	0.43±0.05	0.41±0.13	0.51±0.14

Table 6. Relative organ weight (organ to body weight ratio) of female mice which were observed at day 14th after treated with a single oral dose of EtOAc extract from irradiated tea parasite

Test group (dose per kg BW)	Relative organ weight (organ weight to body weight ratio)					
	Liver		Liver		Liver	
Control (0 g)	4.64±0.38	0.58±0.27	0.93±0.08	0.59±0.07	0.45±0.04	0.54±0.43
Dose 1 (0.625 g)	5.04±0.96	0.65±0.27	0.99±0.34	0.88±0.77	0.47±0.09	0.23±0.13
Dose 2 (1.25 g)	4.81±0.68	0.50±0.24	0.98±0.17	0.58±0.08	0.43±0.05	0.34±0.25
Dose 3 (2.5 g)	4.31±0.57	0.79±0.48	0.98±0.15	0.62±0.15	0.47±0.10	0.40±0.30
Dose 4 (5 g)	4.47±0.70	0.57±0.23	0.96±0.18	0.59±0.06	0.40±0.09	0.41±0.33
Dose 5 (10 g)	5.20±0.70	0.61±0.19	0.92±0.24	0.66±0.13	0.55±0.25	0.33±0.18

4. CONCLUSIONS

It can be concluded that no acute toxicity was found in mice treated with a single oral dose of EtOAc extract from unirradiated and irradiated tea parasite at the dose of 10 kGy. At the dose of EtOAc extract up to 10 g/kg (equivalent to 77.6 g of extract which administered to human), the normal body weight gains were observed in mice of all dose groups, no mice deaths in any of the dose groups, and no significant change in organ weight relative to body weight.

The approximate lethal dose for male and female mice were determined to be higher than 10 g/kg of mice body weight.It is suggested that the treatment of EtOAc extract from unirradiated and irradiated tea parasites until dose up to 10 g/kg of mice body weight was still safe.

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