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The effects of *Eucheuma cottonii* on sperm quality and tissue lead level in rats exposed to lead nitrate

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ABSTRACT

The aims of this study were to determine the capability of *Eucheuma cottonii* to improve sperm quality and to reduce lead accumulation in organs of rats exposed to lead. Sprague Dawley rats (n=24) were randomly assigned to four groups. Group A served as a control and received maintenance diet, Group B received maintenance diet and supplemented with 500 $mg.kg^{-1}$ of *E. cottonii*, Group C received maintenance diet and 20 $mg.kg^{-1}$ lead nitrate and Group D received maintenance diet, 500 $mg.kg^{-1}$ *E. cottonii* and 20 $mg.kg^{-1}$ lead nitrate. Rats were fed with the designated diet for five weeks and bodyweight was monitored on weekly basis. Sperm quality was evaluated manually and lead accumulation in internal organs was measured by atomic absorption spectrophotometer. Lead intoxication significantly reduced bodyweight and sperm quality. *E. cottonii* supplementation in healthy rats significantly increased sperm quality and reduced bodyweight. Meanwhile, *E. cottonii* supplementation to lead-exposed rats was able to improve their bodyweight and decrease lead accumulation in rat's internal organs. In addition, sperm quality in lead-exposed rats improves significantly following *E. cottonii* supplementation. We conclude that *E. cottonii* efficiently increased sperm quality in healthy and lead-exposed rats. *E. cottonii* also possess a capability as a chelating agent.

KEYWORDS

Eucheuma cottonii;
Lead;
Chelating;
Sperm quality.

INTRODUCTION

The toxic effects of lead have long been recognized and to date remain a major public health problem[1]. Human exposure to lead occurs through various sources like leaded gasoline, industrial processes, lead-based paints, lead containing pipes, battery recycling and others[2]. Lead intoxication results in various behavioral and physiological disorders. Chronic and acute lead intoxication causes cardiac and vascular damage, leading to

hypertension and cardiovascular diseases[3]. It also negatively affect the lifespan of red blood cells and restraining the synthesis of hemoglobin, therefore, leads to anemia[4]. Neil and co-workers[5] reported that lead toxicity may induce retinal cell apoptosis and loss of lens transparency.

Lead also act as gonad toxicants and correspond to different compounds related to social habits, life conditions, work hazards or the use of drugs and medicines[6]. It become a male reproductive toxicant by disrupting the hypothalamic control of pituitary hormone secretion, reduced libido, increase the number of abnormal sperm and gamete chromosomal damage[7]. A case study in Finland showed a connection between paternal exposure to lead and birth defects in their children[8]. Meanwhile, in female reproductive system, lead intoxication causes infertility, miscarriage, premature membrane rupture, pre-eclampsia, pregnancy hypertension and premature delivery[7].

The fact is, once lead enters the body, it is almost impossible to remove it completely or to reverse its damages effects; therefore, preventive measures are preferred over the treatment. Liver tissue is the largest repository of lead among soft tissues followed by kidneys[9]. Currently, lead poisoning are treated with drugs designated as chelating agents by chelation therapy. Chelation therapy is an injection of chelating agent such as dimercaptosuccinic acid (DMSA) or ethylene-diamine-tetra acetic acid (EDTA) to remove heavy metals from the body by urination. The major drawback in the usefulness of certain chelating agents is their side effects such as irregular heartbeat, kidney damage and anemia if used inappropriately[10].

Recently, there is a greater global interest in non-synthetic, natural drugs derived from herbal sources due to their better tolerance and minimum adverse drug reactions[11]. Toxicity of lead is mainly attributed to the induction of oxidative stress by elevation of reactive oxygen species (ROS), therefore increased interest among phytotheraphy investigators to use plants material with anti-oxidant activity for protection against lead intoxication[12]. Jackie and co-workers[13] reported that supplementation of *Etlingera elatior* increased total antioxidants and antioxidant enzyme levels in rats exposed to lead acetate. As documented, the latex of *Ficus carica* possessed a significant hepatoprotective activity against lead-induced hepatotoxicity in rats[12]. *Spirulina fusiformis* prevented lipid peroxidation and restored levels of endogenous antioxidants to normal in liver, lung, heart and kidney of lead-exposed animals[1].

Eucheuma cottonii is a species of red seaweed or Rhodophyta. It is abundantly cultivated in South East Asia, with potential in Africa and the Pacific Islands[14]. *Eucheuma cottonii* is an edible species and rich with fibers, vitamins, proteins, minerals, fatty acids, polyphenols as well as antioxidants[15,16]. In view of this consideration, the functional health effect of *Eucheuma cottonii* in protecting against lead toxicity would be of current interest. Therefore the current study was designated (i) to evaluate the effect of *Eucheuma cottonii* on sperm quality of rats exposed to lead, and (ii) to test the potential of *Eucheuma cottonii* to reduce organ lead residues in experimented lead-exposed rats.

MATERIALS AND METHODS

Plants material

Eucheuma cottonii was bought from local market around Selangor, Malaysia and was authenticated by an expert at the Herbarium, Universiti Kebangsaan Malaysia.The voucher specimen (AI-1001) was deposited in the research laboratory at Universiti Teknologi MARA. The dried *E. cottonii* was washed under running tap water followed by distilled water. Then it was cut into small pieces, before 5000 g of *E. cottonii* was soaked in 1000 ml distilled water for 2 days. The mixture of *E. cottonii* and distilled water was then blended using commercial blender and kept in 20 ^{0}C until use.

Animals

Male Sprague Dawley rats weighing approximately 250-300 g were purchased from Universiti Malaya Animal House. All animals were treated in accordance with the principles of laboratory animal care. They were housed in polypropylene cages in an air conditioned room with temperature maintained at 25±3 ^{0}C, relative humidity of 50±5 % and 12 hours alternating light/dark cycle. The rats had access to an animal diet and tap water *ad libitum*.

Group of treatments

After acclimatization to laboratory conditions,

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Sprague Dawley rats were divided into 4 groups of 6 rats each. Animals were given rat chow and tap water *ad libitum* as a maintenance diet. In addition, they were treated by oral gavage as follows;

Group A: Control (1 ml of distilled water)

Group B: Rats supplemented with *Eucheuma cottonii* (500 mg.kg^{-1} of bodyweight)

Group C: Rats exposed to lead acetate (20 mg.kg^{-1} of bodyweight)

Group D: Rats exposed to lead acetate (20 mg.kg^{-1} of bodyweight) and supplemented with *Eucheuma cottonii* (500 mg.kg^{-1} of bodyweight)

All treatments were given daily for 5 weeks. Distilled water, *E. cottonii* and lead acetate were given every morning in between 9 to 10 am.

Body weight measurement

Changes in bodyweight were recorded on a weekly basis using a precision scale (Kern & Sohn, Germany).

Sperm quality evaluation

The cauda epididymis was dissected, minced and incubated in a pre-warmed petri dish containing Toyoda-Yokoyama-Hosi (TYH) medium[17]. The sperm was allowed to disperse into the buffer at 37 ^{0}C and 5% $C0_2$ in CO_2 incubator. After 10 minutes, the debris tissue of cauda epididymis was removed and the sperm suspension was incubated again in CO_2 incubator for another 10 minutes.

The sperm count, morphology and mortality of the sperm were determined manually[18]. The sperm suspension was analysed for sperm count by Makler Counting Chamber (Sefi-Medical Instruments, USA). Approximately 5 µl of the sperm suspension was transferred into a Makler Chamber. Then the settled sperm were observed and counted under light microscope (Olympus Model CH30, Japan).

As for sperm morphology evaluation, a drop of sperm suspension was smeared on a clean glass slide. The smear was then air dried and fixed in a methanol for five seconds. Then the slide with sperm smear was stained with Giemsa stain solution for 30 minutes. Stained slide was then observed under light microscope (Olympus Model CH30, Japan) to assess for morphological abnormalities sperm head, midpiece and tail.

Sperm mortality was evaluated by one-step eosin-nigrosin staining technique[19]. Equal volumes of sperm suspension (50 µl) and eosin-nigrosin stain (50 µl) were mixed. The mixture was incubated for 30 second at room temperature. Then, a droplet (12 µl) of mixture was transferred with the pipette to a labeled microscope slide where it was smeared by sliding a cover slip in front of it. The smear was air dried and examined under light microscope (Olympus Model CH30, Japan). Sperm that were white or unstained were classified as live sperm and those that showed any pink or red colouration were classified as dead sperm.

Estimation of lead accumulation by atomic absorption spectrophotometer

Samples of blood, liver, kidney and testis were collected separately for measurement of lead concentration. Tissues were digested and lead contents were measured using an atomic absorption spectrophotometer (Shimadzu Model AA-670, Japan) as previously described[20].The tissue was homogenized with pestle and mortar. Then, 5 g of the tissue was digested with 10 ml of 65% nitric acid. After that, the mixture was heated until the mixture became colourless, before allowed to cool at room temperature. The mixture then was made up to 25 ml by adding deionised water and stirred. Later, the mixture was filtered by using Whatman Filter Paper No. 41. A lead content was measured using an atomic absorption spectrophotometer at a wavelength of 217 nm.

Statistical analysis

ANOVA (SPSS 18th Edition) was used to detect and locate the effects of *Eucheuma cottoni* on sperm quality and lead accumulation in healthy and lead-exposed rats. All results were considered statistically significant when $p<0.05$ and values are reported as a mean ± s.e.m.

RESULTS AND DISCUSSION

Live bodyweight

At the start of the experiments, rats from each group had a similar bodyweight (Figure 1). Over 5 weeks of treatments, all rats increased their live bodyweight, indicating that the dietary treatments allowed the growth of the animals. However, the live

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bodyweight of Group C (rats exposed to lead) significantly ($p<0.05$) lower, compared to rats in Group A (control; maintenance diet). Meanwhile the rats supplemented with *E. cottonii* (Group B) also showing live bodyweight lower than rats in Group A (control group) but higher than rats in Group C (rats exposed to lead). However, the differences were not statistically significant ($p>0.05$). When lead-exposed rats supplemented with *E. cottonii* (Group D), their live bodyweight improve over Group C (rats exposed to lead without *E. cottonii* supplementation).

The significant reduction in growth rate of lead intoxicated animals in the current study perhaps associated with the capability of lead to disturb metabolic activity as previously reported[6]. Meanwhile several authors[21] suggested that the lower growth rate in lead intoxicated animals was due to reduced food consumption via lead effects on the satiety set-point. However in the current study, we do not measure food consumption and water intake in each group.

The slow rate of body growth in Group B (rats supplemented with *E. cottonii*) compared to Group A (control; maintenance diet) suggests that *E. cottonii* possess anti-obesity materials. A similar result reported by the previous authors[22] that *E. cottonii* inhibit bodyweight gain in high-cholesterol/high-fat diet rats. According to Jeon and co-workers[23], nutrients-rich seaweed suppresses bodyweight gain and has a potential as an anti-obesity agent.

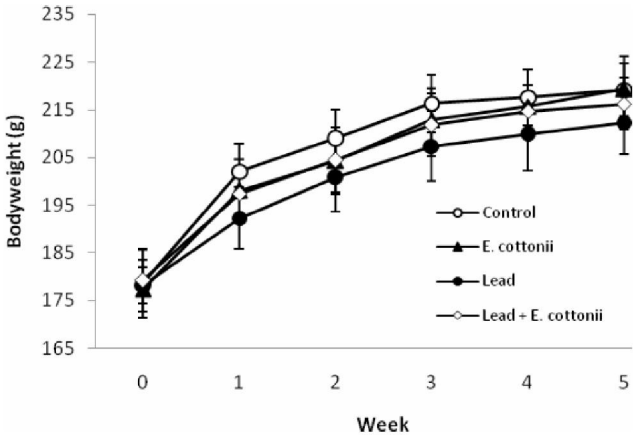

Figure 1: Live bodyweight of male rats fed with maintenance diet (open circles), supplemented with *E. cottonii* (closed triangles), exposed to lead nitrate (closed circles) and combination of *E. cottonii* and lead nitrate (open squares) over 5 weeks of experiment.

Interestingly, *E. cottonii* supplementation improves live bodyweight in rats exposed to lead. We postulated that *E. cottonii* reduce the lead effects on the satiety set-point. The addition of *E. cottonii* also increases metabolic activity in lead-exposed rats, because *E. cottonii* rich with antioxidant properties and other pharmacologically important bioactive constituents[16,17,24] that is essential to enhance animals and human health.

Blood and tissues lead level

Eucheuma cottonii supplementation significantly ($p<0.05$) reduced tissue lead accumulation in Group D (rats exposed to lead + *E. cottonii* supplementation) compared to Group C (rats exposed to lead) (TABLE 1). The mechanism by which *E. cottonii* could reduce lead accumulation are unknown. The efficiency of *E. cottonii* was perhaps due to the presence of biologically active compounds that prevent absorption of lead from gastro-intestinal tract or enhanced lead excretion from the body. Previous authors[25,26] documented that the presence of sulphur containing amino acids in plant materials, or biological compounds having free carboxyl (C=O) and amino (NH_2) groups might have heavy metal chelating activities. Further research is now on-going to confirm the presence of this type of compounds, followed by isolation of chelating agent bio-compounds in *E. cottonii*. In addition, it was observed that in the current study, a large amount of lead deposition was found in the liver and kidney, and this finding is in agreement

TABLE 1: Lead concentration in blood and organs of rats fed with maintenance diet; control (Group A), supplemented with *E. cottonii* (Group B), exposed to lead nitrate (Group C) and concurrent exposure to lead nitrate and *E. cottonii* supplementation (Group D). Values are mean±s.e.m.

	A	B	C	D
Liver (µg/g	0.0036±0.000E	0.0042±0.000E	0.3021±0.0007*	0.2341±0.0009**
Kidney(µg/g)	0.0032±0.000E	0.0038±0.000E	0.3042±0.0005*	0.2563±0.0007**
Blood(µg/ml)	0.0038±0.000E	0.0036±0.000E	0.3165±0.0008*	0.2075±0.0004**
Testis (µg/g)	0.0024±0.000E	0.0028±0.000E	0.2781±0.0003*	0.1981±0.0006**

*p<0.05 compared to Group A (normal; maintenance diet); **p<0.05 compared to Group C (rats exposed to lead).

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with the previous authors[9]. This is not a surprised since liver and kidney is a vital organ for detoxifying and eliminating heavy metal such as lead.

Sperm quality

In parallel with the reduction of lead level in the testis, the sperm quality improved in Group D (rats exposed to lead + *E. cottonii* supplementation) compared to Group C (rats exposed to lead) (TABLE 2). Sperm quality also increased in Group B (rats supplemented with *E. cottonii*) compared to Group A (control; fed with maintenance diet). Meanwhile, rats in Group C (rats exposed to lead) decreased their sperm quality over other groups.

With regards to sperm count, Group C (rats exposed to lead) significantly ($p<0.05$) decreased their sperm concentration by 53% compared to Group A (control; maintenance diet). On the other hand, Group B (rats supplemented with *E. cottonii*) significantly ($p<0.05$) increased their sperm count by 64% over Group A (control; maintenance diet). It was noticed that, *E. cottonii* supplementation significantly ($p<0.05$) improved sperm count in Group D (rats exposed to lead + *E. cottonii* supplementation) by 48% over Group C (rats exposed to lead).

Sperm mortality was increased by 30.1% in Group C (rats exposed to lead) compared to Group A (control; maintenance diet). Interestingly, there was improvement in sperm survivability following *E. cottonii* supplementation. Healthy rats supplemented with *E. cottonii* (Group B) decreased their sperm mortality by 7.4% compared to control received maintenance diet (Group A). Meanwhile in Group D (rats exposed to lead + *E. cottonii* supplementation), sperm mortality was decreased by 24.2% compared to Group C (rats exposed to lead).

TABLE 2 : Sperm quality of rats fed with maintenance diet; control (Group A), supplemented with *E. cottonii* (Group B), exposed to lead nitrate (Group C) and concurrent exposure to lead nitrate and *E. cottonii* supplementation (Group D). Values are mean±s.e.m

	A	B	C	D
Sperm count (10^6/ml)	53.2 ± 1.7	86.9 ± 1.5*	23.6 ± 1.8*	44.3 ± 1.6**
Mortality (%)	17.7 ± 1.3	10.3 ± 1.7*	47.8 ± 2.7*	23.6 ± 2.1**
Abnormality (%)	5.9 ± 0.3	5.4 ± 0.7	37.6 ± 2.9*	17.1 ± 1.3**

***p<0.05 compared to Group A (normal; maintenance diet); **p<0.05 compared to Group C (rats exposed to lead)**

There were no significant ($p>0.05$) difference in sperm morphology between Group A (control; maintenance diet) and Group B (rats supplemented with *E. cottonii*). However the number of abnormal sperm was increased significantly ($p<0.05$) in Group C (rats exposed to lead) compared to Group A and Group B. When lead-exposed rats been supplemented with *E. cottonii* (Group D), the number of abnormal sperm decreased significantly ($p<0.05$) by 20% compared to Group C (rats exposed to lead).

The generation of ROS (reactive oxygen species) due to lead exposure was associated with loss of motility, sperm abnormality, disruption of membrane integrity and impaired mitochondrial function in spermatozoa[27]. Normally, male reproductive system produced certain amount of superoxide dismutase, catalase and some reductases as a defence mechanism against ROS[28]. The addition of the *E. cottonii* to the rat's diet in the current experiment probably improves the defence mechanism against oxidative stress in rat's reproductive system. *E. cottonii* has been demonstrated to have strong antioxidant property[29]. Therefore, *E. cottonii* supplementation probably increases the production of superoxide dismutase and other enzymes in male's reproductive system against ROS. In addition, antioxidant properties in *E. cottonii* possess the peroxyl radicals scavenging capability[29], thus capable to increase sperm quality in healthy and lead-exposed rats.

CONCLUSION

In accordance with the obtained results, we concluded that *Eucheuma cottonii* possess a potential as an alternative chelating agent by reducing lead accumulation in internal organs. *E. cottonii* also has a potential as a fertility promoter in both, the healthy rats and the one exposed to lead.

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