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Research Article

The Protective Efficacy of Ethanolic Leaves Extract of Citronella Gras (*Cymbopogon nardus*) on Glucose Metabolism Alteration Induced By Mercury in Rats

Fransisca Diana Alexandra[1*], Dian Mutiasari[2], Trilianty Lestarisa[2], Eko Suhartono[3]

[1]*Department of Medical Pharmacology, Faculty of Medicine, Palangkaraya University, Central Kalimantan, Indonesia*
[2]*Department of Public Health, Faculty of Medicine, Palangkaraya University, Central Kalimantan, Indonesia*
[4]*Department of Medical Chemistry/Biochemistry, Faculty of Medicine, Lambung Mangkurat University, Banjarmasin, Indonesia*

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ABSTRACT

The present study was undertaken to investigate the protective effect of ethanolic citronella grass (C. nardus) leaves extract against mercury (Hg) induced glucose metabolism alteration in rats. Four groups of rats were selected, with 6 rats for each group. Animals of group I was received a 1 ppm of Hg only. Animals of groups II, III, and IV received a combination of 1 ppm Hg and plant extract in different dose (1650, 2520, and 3360 mg/ml). The experiment lasted for 4 weeks. The various parameters studied included liver weight, liver glucose, glycogen, and malondialdehyde (MDA) level in all groups after treatment. The results of this present studies showed that the Hg-induced glucose metabolism alteration in rats which can be seen from the increase of liver glucose and the decreasing of liver glycogen levels. The results also showed that the Hg-induced glucose metabolism alteration through its activities in the trigger the liver cells damage which can be seen from the decreasing of liver weight and the increase of liver MDA level. The ethanolic of C. nardus leaves extract shows a protective effect to maintain all parameters into a better a condition which can be seen from the significant increase in liver weight and liver glycogen level, and the significant decrease in liver glucose and MDA levels. The present study indicated that the ethanolic C. nardus leaves extract showed a potential protective effect on glucose metabolism alteration induced by Hg.

Keywords: *C. nardus*, Citronella grass, Glucose, Glucose Metabolism.

INTRODUCTION

Mercury (Hg) is a highly toxic element, which we often see discussed together with cadmium and lead, which are prominent examples of toxic heavy elements[1]. This metal known has no biological function. Its release can occur through natural sources such as volcanic activities or anthropogenic activities such as industrial processes, agriculture, and mining, consequently making human exposure to Hg almost impossible to avoid[2].

Hg has three valence states and exists in several forms: inorganic mercury, which includes liquid metallic-Hg and Hg-vapor (Hg^0), mercurous (Hg^+) and mercuric (Hg^{++}) salts, and organic-Hg, with methyl-Hg (CH_3Hg, MeHg), ethyl-Hg (C_2H_5Hg, EtHg), and phenyl-Hg (C_6H_5Hg, PhHg)[3]. The source, biological properties, and toxicity between these forms of mercury are differed[4]. These differences makes each form of Hg has specific characteristics and effects for target organs. For example, exposure to Hg vapor as well as to organic Hg compounds specifically affects the central nervous system, while kidneys, liver and gastrointestinal tract are mainly targeted by inorganic Hg compounds, such as Hg-chloride ($HgCl_2$)[5].

The liver is pivotal organs of the body responsible for maintaining the homeostasis of the body, which acts as a center of metabolism and detoxification. Accumulations of the chemical pollutants such as Hg is known to adversely affect the histology and functioning of liver[6-7]. In this context, the presence of Hg in liver cells will lead to a broad range of physiological, biochemical, and behavioral dysfunctions, including several metabolism pathways, such as glucose metabolism. Several previous studies were indicated that Hg exposure was affected the levels of glucose both in human and experimental animals[8-10]. Several mechanisms have been proposed for these toxicity effects like oxidative stress mechanism, and the disturbances in glucose utilization which eventually disrupt the overall of these metabolic pathways[10].

Recently, much attention has been focused on the use of antioxidants, especially natural antioxidants. Natural antioxidants exhibit a wide range of pharmacological activities and have been shown to have anticancer, anti-inflammatory and anti-aging properties. Numerous vegetables, crops, spices and medicinal herbs have been tested in an effort to identify new and potentially useful antioxidants[11-12]. Tropical and subtropical regions of the world like Indonesia contain a vast source of natural

**Author for Correspondence: esuhartono@unlam.ac.id*

products, some of which may have the potential to be developed into new drugs for treatment various diseases like citronella grass (*Cymbopogon nardus*; *C. Nardus*)[12-13]. *C. Nardus* is a plant native to Indonesia[14]. Locally, this plant known as 'Serai Wangi'[15]. These species are used for the production of citronella oil, which is used in soaps, as an insect repellent (especially mosquitoes) in insect sprays and candles, and in aromatherapy[14]. Furthermore, according to the previous study, these plant extract and their essential oils are known for their biological activities such as antimicrobial, antiacetylcholinesterase, and antioxidant activities[13].

However, no data are available in the literature of leaves of *C. Nardus* on the alteration of glucose metabolism induced by Hg. Therefore we undertook the present investigation to examine the protective effects of ethanolic leaves extract of *C. Nardus* on glucose metabolism alteration induced by Hg.

MATERIAL AND METHODS

Collection and Identification of Plant materials

The fresh leaves of *C. nardus* were collected from Tangkiling Village, Central Kalimantan, Indonesia. The plant was authenticated by Department of Biology, Palangkaraya University, Central Kalimantan, Indonesia. Before use, it were ensured that the leaves was free from contamination, sand and no microbial growth. The leaves were shade dried and were made into coarse powder using a commercial blender.

Preparation of Extracts

Four portions of 5 g dried *C. nardus* leave powder (oven- and freeze-dried) were weighed using an analytical balance. With the sample to solvent ratio fixed at 1:10, different concentrations of ethanol (v/v; 0%, 10%, 20%, and 30%) were prepared. The mixtures were shaken for 60 min at 25°C and 150 rpm in a shaking incubator. After the extraction, the extracts were filtered using Whatman No. 1 filter paper. The filtrate residue was collected and centrifuged at 4500 rpm for 10 min. The supernatant was concentrated using rotary evaporator at 40°C. The concentrated extract was freeze-dried, wrapped with aluminium foil, and stored at -20°C until further analysis.

Experimental animals

The present study depended on materials got from 24 grown-up male albino rats (*Rattus novergicus*) with the body weight from 200-250 g with 2-3 months old. They were purchased from the Abadi Jaya farm at Yogyakarta, Indonesia, in healthy condition. They were allowed to acclimatize for at least 2 weeks before treatment. At the acclimatization period, the rats were separated into 4 groups of 6 animals each. The animals were kept in a room with a photoperiod dark light cycle 12:12 and 28±2˚C temperature. Every group of animals kept in the same traditional state of eating regimen and environment. Consideration and treatment of animals were performed by according to the approval of ethics regulation at Faculty of Medicine, University of Lambung Mangkurat, Banjarmasin, South Kalimantan, Indonesia.

Experimental design

The experiment lasted for 4 weeks. The rats were randomly separated into 4 groups of 6 animals each groups. The groups are:

Group 1 (control; C): Hg-chloride ($HgCl_2$) treated rats (1 ppm $HgCl_2$) (n =6)

Group 2: *C. nardus* ethanolic leaves extract (1650 mg/ml) + $HgCl_2$ (1 ppm $HgCl_2$) (n =6)

Group 3: *C. nardus* ethanolic leaves extract (2520 mg/ml) + $HgCl_2$ (1 ppm $HgCl_2$) (n =6)

Group 4: *C. nardus* ethanolic leaves extract (3360 mg/ml) + $HgCl_2$ (1 ppm $HgCl_2$) (n =6)

All treatment was administered by the oral route (gavage), in a single daily dose at 10:00 h in the morning. After the treatment, the animals fasted overnight. After overnight starvation, the liver sample of each animal was quickly gathered under ether anesthesia. Then, each liver sample was carefully weighed. After weighing, the samples were immediately cleaned and washed with the phosphate buffer solution. The cleaned samples were immediately extracted, washed in super cold saline to clear from the blood, finely minced in the same arrangement and homogenized. The homogenates were centrifuged at 3500 rpm for 10 min, and the supernatants were utilized for liver glucose, glycogen and MDA level. All experimental models and measurement were done in Medical Chemical/Biochemical Laboratory, Faculty of Medicine, Lambung Mangkurat University, Banjarbaru, South Kalimantan, Indonesia.

Estimation of liver glucose concentration

Liver tissues were homogenized in 50% Trichloroacetic Acid (TCA), keeping the proportion of 100 mg per 1.0 ml of TCA. After centrifuging for 5 min at 5000 rpm, the contents of glucose were determined in the supernatant. Homogenate samples were submitted to the same procedure, keeping the same proportions (100 µl of homogenate/1.0 ml TCA). Glucose was determined by Dubois hydrolytic method. It consists of a suitable aliquot of glucose into a final volume of 0.5 ml added of 0.7 ml of 3% phenol. After shaking, 2 ml of concentrated sulfuric acid (H_2SO_4) was added into one stroke developing strong heat of reaction. The product was determined at 540 nm in a single colorimeter[16].

Estimation of liver glycogen concentration

This assay was performed as described by Bidinotto *et al.*[16] Samples of liver were quickly separated from freeze tissues and transferred to assay tubes containing 1.0 ml of 6 mol/l potassium hydroxide (KOH). The tubes were transferred to a boiling water bath and left along 3-5 min for complete dissolution. Aliquots of the resultant solution (250 µl) were added to 3 ml of 95% ethanol-water and after mixing, 100 µl of 10% potassium sulfate (K_2SO_4) was appended. A cloudy white precipitate was formed and the supernatant was discharged after centrifuging at 3000 rpm for 3 min. It was added 2.5 ml of distilled water to the precipitate, which was promptly dissolved. Suitable aliquots of such solution were employed to Dubois reaction. Glycogen concentration is expressed in µmol of glucosil-glucose per g of wet tissue.

MDA concentration analysis

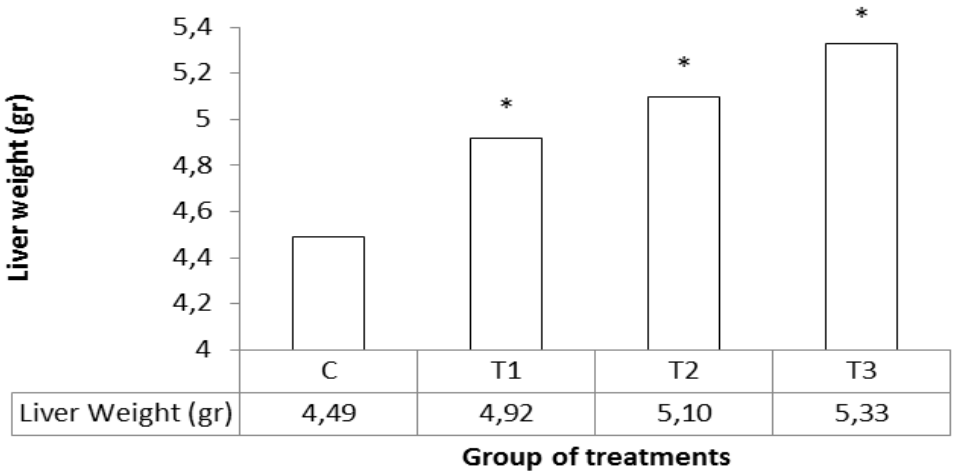

Figure 1: Comparison of liver weight between group oftreatments. C: 1 ppm $HgCl_2$; T1: 1 ppm $HgCl_2$ + of 1650 mg/ml *C. nardus* ethanolic leaves extract; T2: 1 ppm $HgCl_2$ + of 2520 mg/ml *C. nardus* ethanolic leaves extract; and T3: 1 ppm $HgCl_2$ + of 3360 mg/ml *C. nardus* ethanolic leaves extract. Statistical significance *p<0,05 in comparison with control group,

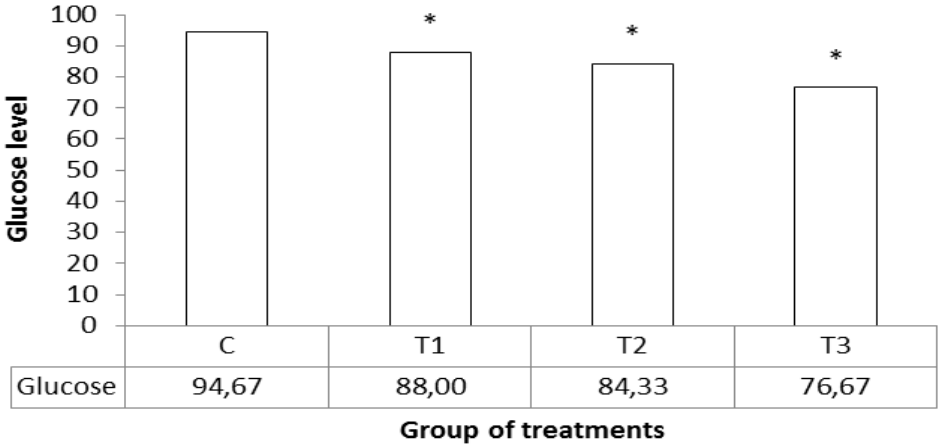

Figure 2: Comparison of liver glucose level between group oftreatments. C: 1 ppm $HgCl_2$; T1: 1 ppm $HgCl_2$ + of 1650 mg/ml *C. nardus* ethanolic leaves extract; T2: 1 ppm $HgCl_2$ + of 2520 mg/ml *C. nardus* ethanolic leaves extract; and T3: 1 ppm $HgCl_2$ + of 3360 mg/ml *C. nardus* ethanolic leaves extract. Statistical significance *p<0,05 in comparison with control group,

MDA levels was calculated by thiobarbituric acid reactive substances (TBARS) by the technique already proposed by Buege and Aust[17-19]. The supernatant were put into a pyrex tube that contained 10% of trichloracetic acid and 0.67% of thiobarbituric acid and incubated at 100ºC for 15 min. Then chill the mixture on ice for 5 min and add the 1.5 ml of n-butyl-liquor. Let the mixture stand for 40 s and centrifuged at 1000 rpm for 15 min. The TBARS value was calculated by the spectrophotometer at the absorbance of 532 nm and figured utilizing the coefficient 1.56 × 105 mol/cm. The MDA concentration expressed in μmol MDA. As a standard solution we used commercially MDA.

Data Analysis

The results were expressed as mean ±SE for three replicates. Significance of mean differences of all parameters between treatment and control groups at the same time and between different time of Cd exposure were statistically compared using two-way Analysis of Variance (ANOVA) and followed by a post hoc Tukey's Honestly Significant Difference (HSD) test for multiple range test. The software used for the data analysis were the Statistical Package for the Social Sciences (SPSS) version 17.0 and Microsoft Excell 2007 for Windows Vista.

RESULTS

Liver weight

Figure 1 shows the liver weight of control and experimental animals in each group. The mean liver weight was significantly increased ($p<0.05$) in all group of treatments as compared to control. The liver weight was found to be increased in *C. nardus* ethanolic leaves extract in a concentration-dependent manner. Extract at a concentration of 3360 mg/ml was found to be most effective

Liver glucose level

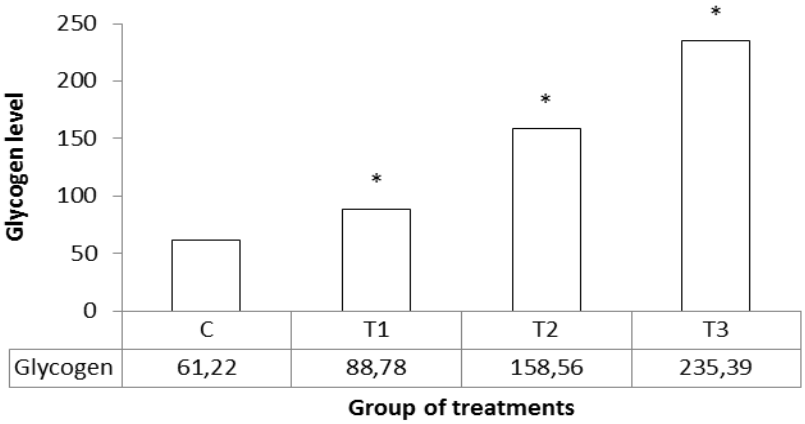

Figure 3: Comparison of liver glycogen level between group oftreatments. C: 1 ppm $HgCl_2$; T1: 1 ppm $HgCl_2$ + of 1650 mg/ml *C. nardus* ethanolic leaves extract; T2: 1 ppm $HgCl_2$ + of 2520 mg/ml *C. nardus* ethanolic leaves extract; and T3: 1 ppm $HgCl_2$ + of 3360 mg/ml *C. nardus* ethanolic leaves extract. Statistical significance *p<0,05 in comparison with control group,

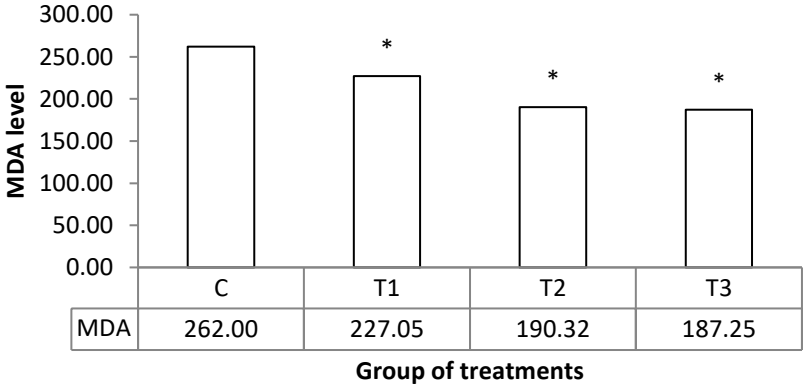

Figure 4: Comparison of liver MDA level between group oftreatments. C: 1 ppm $HgCl_2$; T1: 1 ppm $HgCl_2$ + of 1650 mg/ml *C. nardus* ethanolic leaves extract; T2: 1 ppm $HgCl_2$ + of 2520 mg/ml *C. nardus* ethanolic leaves extract; and T3: 1 ppm $HgCl_2$ + of 3360 mg/ml *C. nardus* ethanolic leaves extract. Statistical significance *p<0,05 in comparison with control group,

Figure 2 shows the liver glucose level of control and experimental animals in each group. The mean liver glucose level was significantly decreased ($p<0.05$) in all group of treatments as compared to control. The liver glucose level was found to be increased in *C. nardus* ethanolic leaves extract in a concentration-dependent manner. Extract at a concentration of 3360 mg/ml was found to be most effective

Liver glycogen level

Figure 3 represented the mean values±standard error (mean±SE) of liver glycogen concentration. Dispersion of measured values around each mean varied from 61.22 to 235.39. Treatment with Hg (control group) led to lower liver glycogen concentrations than another group of treatments. That data also suggests the highest liver glycogen concentration is on T3 group and the lowest was the C group. Statistical analysis test results show that all groups of treatment significantly ($p<0.05$) increased liver glycogen concentration compare to control. The data from figure 3 also suggest that T3 group is the most effective to maintain the liver glycogen concentration than the other treatment group.

Liver MDA level

Figure 4 represented the mean values±standard error (mean±SE) of liver MDA concentration. Dispersion of measured values around each mean varied from 187.25 to 262.00. Treatment with Hg (control group) led to higher liver MDA concentrations than another group of treatments. That data also suggests the lowest liver MDA concentration is on T3 group and the highest was the C group. Statistical analysis test results show that all groups of treatment significantly ($p<0.05$) reduced liver MDA concentration compare to control. The data from figure 4 also suggest that T3 group is the most effective to maintain the liver MDA concentration than the other treatment group.

DISCUSSION

To best of our knowledge, this present study is the first report showing the protective effect of *C. nardus* ethanolic leaves extract on Hg-induced glucose metabolism disturbance in rats. It is well documented that Hg is a toxic substance which can as well be regarded as an important factor in hepatotoxicity.[20] To see the toxic effects of the Hg

on the liver, in this present study the liver weight was measured. According to the result, it seems Hg exposure appears to decreased the liver weight. This research result is quite interesting because some of the results of other studies have shown different results. Ezejindu and Chinweife[21] result study shows that there was a relative increase in liver weight for the mercury exposed animals compared to another group which is treated with distilled water, extract of *Rauwolfia vomitoria* only, and extract of *Rauwolfia vomitoria* + Hg. The increasing of liver weight by Hg is also supported by the results of Okekeani et al.[22] research who found the same results but using different plant extracts, which is the leaves of *Ocimum gratissimum*. On the other hand, Haouem et al.[20] result study did not observe any change in the ratio of liver weight to body weight in rats treated with $HgCl_2$. These different results may be caused by several things, among others; (1) In this study there was no negative control group that was not exposed to mercury, so it is difficult to know clearly whether the liver weight higher or lower; (2) Hg exposure may cause an atrophy, necrosis or cirrhosis of liver cells, so the liver weight seems to be lower than another group of treatments; or (3) the different results may be related to the dose and the duration of the experiment. Further investigation might be needed.

Considering the liver is one of the vital organs that serves as the central metabolism, exposure to Hg on the liver, may cause metabolic disturbances[22]. One of the metabolic pathways can be disrupted by Hg exposure is glucose metabolism[23]. The result of this study revealed that in the Hg-exposed group have the highest glucose level and a lowest glycogen level in liver. From this point of view, Hg exposure seems to increase liver glucose level and decrease liver glycogen level. It is thought to be due to enhanced gluconeogenesis and glycogenolysis and decreased glucose utilization under oxidative stress enzyme produced by Hg[10]. Furthermore, Hg could interact with several enzymes that involved in glucose metabolism. Hg could bind to the sulfhydryl (-SH) of enzymes. Thus, Hg will affect the activity of the enzyme[23].

As mentioned earlier, Hg exposure could cause the disturbance of glucose metabolism which was marked by the increasing of liver glucose and the decreasing of liver glycogen levels. From this point of view, if we connect these results with liver weight result, it seems the liver cells may have a damage due to Hg exposure. These result also in line with the result of this present study for liver MDA level. MDA was known as an end product of lipid peroxidation. Reactive oxygen species degrade polyunsaturated fatty acid, forming MDA. This compound is a reactive aldehyde and is one of the many reactive electrophilic species that causes toxic stress in cells and form covalent protein adducts which are referred to as advanced lipoxidation end products[24]. MDA is well known as one of the important biomarkers of oxidative stress[25]. According to this present study result, the liver MDA level seems to be highest in the Hg-exposed group. It indicates that Hg exposure induced oxidative stress which promotes a further reaction to lead to liver cells death and damage.

According to our results, it seems the administration of *C. nardus* ethanolic leaves extract could maintain the disturbance of glucose metabolism induced by Hg. It can be seen from the results of this present study that shows the decreasing of glucose levels and increasing of glycogen levels if Hg treatment is combined with the extract. It is also strengthened by an increase in liver weight if Hg is combined with the extract. This effect is in a dose-dependent manner. The protective effect of the extract may be caused by the phytochemical constituents and antioxidant activity of the extract. Liliwirianis et al.[26] result study indicated that the leaves extract of *C. nardus* contained several phytochemical constituents, such as alkaloid, saponin, phenolic compound, flavonoid, and terpenoid. Another study by Kumar et al.[27] indicated that the leaves extract of *C. nardus* have several specific antioxidant enzymatic and non-enzymatic activities, such as ascorbate peroxidase, catalase, superoxide dismutase, ascorbic acid, a phenolic compounds, and carotenoids. This might be the reason why the extract has the protective effect. This results also strengthened by a decrease in liver MDA level. The liver MDA level seems to be reduced if the Hg is combined with plant extract.

In conclusion, our present investigation shows the protective efficacy of ethanolic *C. nardus* leaves extract on Hg-induced glucose metabolism disturbance in rats. Also, the results of this present studies showed that the most effective efficacy is found in a higher dose. Further studies are undergoing in order to clarify their molecular mechanisms.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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