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AMELIORATIVE EFFECTS OF DIFFERENT PARTS OF *GEMOR* (*Nothaphoebe coriacea*) ON CADMIUM INDUCED GLUCOSE METABOLISM ALTERATION *IN VITRO*

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ABSTRACT

Objective: The present study was undertaken to investigate the ameliorative effect of the bark and leaves of *Nothaphoebe coriacea* (*gemor*) on Cadmium (Cd) induced glucose metabolism alteration in liver homogenate *in vitro*.

Methods: Glucose metabolism alteration in liver homogenate was induced by the administration of Cadmium Sulphate (CdSO₄) at a dose 3 mg/l. Ameliorative effect of the leaves and bark extracts was determined by assessing the concentration of glycogen, glucose and Methylglyoxal (MG). Dubois hydrolytic method were used for liver glycogen and glucose concentration estimation. Modified Dinitro Phenyl Hydrazine (DNPH) method was used for MG concentration estimation.

Results: The results of this present studies showed that treatment with CdSO₄ significantly decreased the levels of glycogen and MG concentration, and increased the level of glucose in liver homogenate compared to control. The aqueous extracts of bark and leaves of *gemor* significantly increased the levels of glycogen and MG concentration, and decreased the level of glucose in liver homogenate compared to control. The aqueous extracts of the bark of *gemor* in comparison with CdSO₄ treatment group showed significant effect to maintain the glycogen, glucose, and MG concentration in liver homogenate. However, when compared to the aqueous extracts of leaves of *gemor* the result was not significant. The results suggest aqueous extracts of the bark of *gemor* was more effective to prevent the glucose metabolism alteration induced by CdSO₄ than the aqueous extracts of leaves of *gemor*.

Conclusion: The present study demonstrated Cd could induced the glucose metabolism alteration in liver homogenate, and the aqueous extracts of bark and leaves of *gemor* showed the ameliorative effect to prevent this alteration. In addition, the bark was more effective than leaves of *gemor* to prevent the glucose metabolism alteration induced by Cd.

Keywords: Cadmium, *Gemor*, Glucose Metabolism, *Nothaphoebe coriacea*.

INTRODUCTION

In recent years, advancement in technology has led to high levels of industrialization leading to the discharge of effluents and emissions containing

heavy metals into the environment [1]. Heavy metals are inorganic elements essential for plant growth in traces or very minute quantities. They are toxic and poisonous in relatively higher

concentrations [2]. These toxic metals can enter the human body by the consumption of contaminated food crops, water or inhalation of dust [3]. Furthermore, this will lead to serious health disorders [4-5]. Among these heavy metals, Cadmium (Cd) is one of the heavy metals that have great environmental health effects [6].

Cd is widely distributed in the environment due to its use in many industries [7]. Cd is absorbed through ingestion by food and water, and inhalation [8]. Once absorbed, Cd irreversibly accumulates in the human body, particularly in kidneys and other vital organs such as the lungs or the liver [9]. One of the basic mechanisms of Cd toxicity is its negative influence on Cd enzymatic systems of cells, owing to the substitution of other metal ions in metalloenzymes and its very strong affinity to biological structures containing sulphhydryl groups (-SH), carboxyl and phosphate [10]. This will lead to a broad range of physiological, biochemical, and behavioral dysfunctions, including several metabolism pathways, such as glucose metabolism.

It has been found that Cd could change glycogen reserves and serum glucose levels by affecting the activities of liver enzymes that have a pivotal role in the glucose metabolism such as gluconeogenesis, glycogenesis, and glycolysis [11]. Results from Bashir *et al.*, [10] study showed that Cd exposure could increase the levels of plasma glucose and decrease hepatic glycogen. Some investigations also showed that heavy metals could decrease the glycogen reserves in fish and invertebrates [12].

Recently, there is global interest in non-synthetic, natural drugs derived from herbal sources due to better tolerance and minimum adverse drug reactions. *Nothaphoebe coriacea* (Family: Lauraceae) commonly known as "Gemor" is one such plant that potentially has medicinal benefits of the parts of the tree

are either leaves, twig, bark. According to the previous study, the bark of *gemor* known to have viral activity for influenza and herpes virus [14]. Arifin *et al.*, [14] studies also showed that the different parts of *gemor* (twig, bark, and leaves) have anti-inflammation activity. It is also reported that the bark, twig, and leaves of *gemor* contain phytochemical components, such as alkaloid, steroid, flavonoid, saponin, triterpenoid, tannins, and phenolic compounds [15]. This might be the reason for all medicinal properties of different parts of *gemor*.

However, no data are available in the literature of bark and leaves of *gemor* on the alteration of glucose metabolism induced by Cd. Therefore we undertook the present investigation to examine the ameliorative effects of bark and leaves of *gemor* on glucose metabolism alteration induced by Cd through *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The fresh leaves and bark of *gemor* were collected from Tumbang Nusa, Central Kalimantan, Indonesia. The plant was authenticated by Dr. Kade Sidiyasa, Technology Research Institute for Conservation of Natural Resources, Balikpapan, East Kalimantan. Before use, it was ensured that the leaves and bark were free from contamination, sand and no microbial growth. The bark and leaves were shade dried and made into coarse powder using a commercial blender.

Preparation of Extracts

Extraction was done by decoction methods [16]. 100 g of shade-dried bark and leaves of *gemor* were weighed and 1000 ml of distilled water was added to it respectively. This mixture was taken in 1000 ml beaker and subjected to heating continuously for 30 min at a temperature of 90°C. Then the mixture was allowed to cool to room temperature and subjected to filtration by

means of vacuum filter. The filtrate so obtained is concentrated, so that all the excess solvent is evaporated in order to get the concentrated extract.

Experimental Protocol

The liver samples were collected from 32 old male rats (*Rattus novergicus*) with 2-3 mo old, weighing 200-250 g. Then liver samples were taken by surgically procedure with ether as an anaesthesia. Then the liver fixed in phosphate buffer at pH 7.0. The liver was ground to form a liquid. Subsequently the solution was taken and centrifuged at 3500 rpm for 10 min and the top layer were taken and stored until it use.

Furthermore, the liver homogenate were prepared to experimental *in vitro* models. Samples divided into into 4 groups (1 control group and 3 treatment groups). Control (C) group: liver homogenate only, Treatment 1 (T1) group : liver homogenate + 3 mg/l of Cadmium Sulphate (CdSO_4), Treatment 2 (T2) group: liver homogenate + 3 mg/l of CdSO_4 + aqueous extracts of bark of *gemor*, Treatment 3 (T3) group: liver homogenate + 3 mg/l of CdSO_4 + aqueous extracts of leaves of *gemor*. Each solutions then incubated at 37°C for 1 hour. After incubation, liver glycogen, glucose, and Methylglyoxal (MG) concentration were estimated.

All animals used and care were in compliance of the Ethics Commission of Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia.

Estimation of Liver Glycogen Concentration

This assay was performed as described by Bidinotto *et al.*, [17]. 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 sulphate (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 from such solution were employed to Dubois reaction. Glycogen concentration is expressed in μmol of glucosil-glucose per g of wet tissue.

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 was 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 [17].

Estimation of Liver Methylglyoxal Concentration

MG compounds are measured using modified Dinitro-Phenylhydrazine (DNPH) method [18]. From each test solution, 0.5 ml solution were taken, and then each solution was divided to 2 tubes with 0.25 ml volume in each tube. The first tube was the sample (A) and the second tube was blank (B) solution. Then 1 ml DNPH were added into each A tube and 1 ml HCl 2.5 mol/l into each B tube. The tubes were incubated for 45 min in room temperature and protected from light, then tubes were shaken with a vortex for 15 min. The next step is added 1 ml of

TCA 20% into each tube (A and B), then the tubes were incubated for 5 min. Tubes were centrifuged for 5 min with 1400 rpm of speed to separate the supernatant. The pellets are centrifuged and washed three times with the addition of 1 ml ethanol-ethyl acetate. The last step were added 1 ml of urea 9 mol/l and incubate the solution for 10 min in 37°C while it was shaken. The solution was centrifuged again for 5 min in 1400 rpm of speed. Then the absorbance of tube A and B were measured at $\lambda = 390 \text{ nm}$ (ΔA). Furthermore, a total of 25 μL of the homogenate was added to 350 μL DNPH (0.1% DNPH in 2 mol/l HCl) and then 2.125 ml distilled water was added. It is incubated for 15 min at 37°C, then 1.5 ml NaOH 10% was added. Absorbance was measured at $\lambda = 576 \text{ nm}$ (A1). MG level was calculated following to equation :

$$\text{MG level (\%)} = (A1 \div \Delta A) \times 100\%$$

RESULTS

Liver Glycogen Concentration

Figure 1 represented the mean values \pm standard error (Mean \pm SE) of liver glycogen concentration. Dispersion of measured values around each mean varied from 275.56 to 347.78. Treatment with Cd (T1 group) led to lower liver glycogen concentrations than other group of treatments. That data also suggest the highest liver glycogen concentration is on C group and the lowest was the T1 group. Statistical analysis test results shows that all groups of treatment significantly ($p < 0.05$) reduce liver glycogen concentration compare to control. Liver glycogen concentration was significantly higher in T2 group and lower in T3 group in comparison with the T1 group. The data from figure 1 suggest that T2 group more effective to maintain the liver glycogen concentration than the T3 group.

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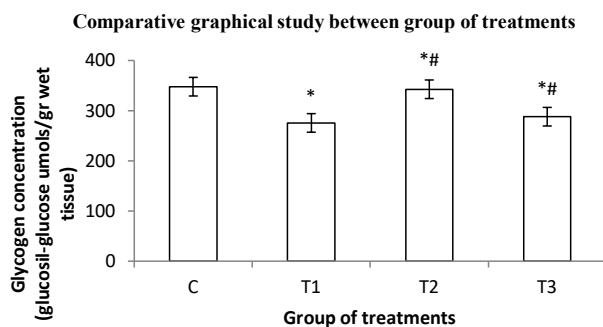


Figure 1. It shows the effect of cadmium and aqueous extracts of leaves and bark of gemor on liver glycogen concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment group.

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Liver Glucose Concentration

Figure 2 represented the mean values \pm standard error (Mean \pm SE) of liver glucose concentration. Dispersion of measured values around each mean varied from 137.00 to 399.45. All groups of

treatment (T1, T2, and T3) showed a higher glucose concentration compare to control. Also, the data from figure 2 shows that treatment with Cd (T1) can increase the glucose concentration compare to control, while treatments with bark (T2)

and leaves (T3) of *gemor* can decrease the glucose concentration compare to T1 group. Statistical analysis test results show treatment with Cd, bark and leaves of *gemor* led to a significant increase of liver glucose levels in comparison with the negative control group. Treatment with a

bark of *gemor* led to significant decrease of the glucose concentration compare with Cd treatment group, and treatment with leaves of *gemor* also decrease the glucose concentration but non-statistically significant.

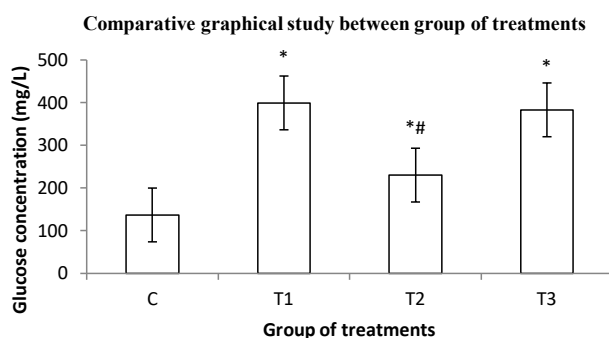


Figure 2. It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver glucose concentration. Values are mean±SEM of four replicates in each groups of treatment. Statistical significance *p<0,05 in comparison with control group, #p<0,05 in comparison with Cd treatment group.

Liver Methylglyoxal Concentration

Figure 3 represented the mean values ± standard error (Mean ± SE) of liver MG concentration. Dispersion of measured values around each mean varied from 15.66 to 69.12. All groups of treatment (T1, T2, and T3) shows a lower MG concentration compare to control. Also, data from figure 3 shows that treatment with Cd (T1) could decrease the MG concentration compare to control, while treatments with bark (T2) and leaves (T3) of *gemor* can increase the MG concentration compare to T1 group. Statistical analysis test results show treatment with Cd, bark and leaves of *gemor* led to a significant decrease of their levels in comparison with the control group. Treatment with a bark of *gemor* led to significant increase of the MG concentration compared with Cd treatment group, and treatment with leaves of *gemor*

also increase the MG concentration but non-statistically significant.

DISCUSSION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, a fight against the disease, nutrient supply, energy provision and reproduction. The major functions of the liver are a carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well-being [19].

The liver may be exposed to large concentrations of exogenous substances and their metabolites [20]. One of the exogenous substances that are harmful to the liver is heavy metal, such as Cd [21].

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The Cd is one of the known environment toxins that is detrimental to liver function on exposure. This will lead to liver cell

damage and will disrupt various metabolic pathways including glucose metabolism [22].

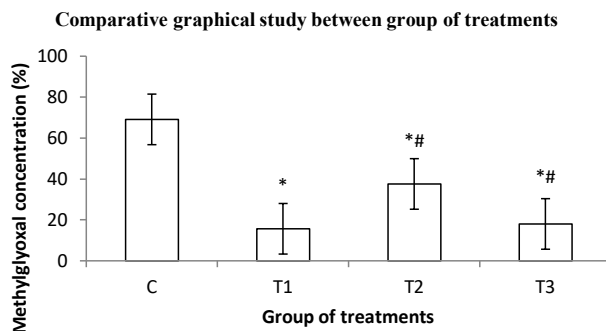


Figure 3. It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver MG concentration. Values are mean±SEM of four replicates in each groups of treatment. Statistical significance *p<0.05 in comparison with control group, #p<0.05 in comparison with Cd treatment group.

Glucose metabolism alteration induced by Cd can be seen from the results of this present study. Cd exposure led to decrease glycogen concentration in liver homogenate. The results of our studies are supported by the other research by Bhati *et al.*, [23]. Results showed Cd could decrease the glycogen level in a liver of rats. Also, Bashir *et al.*, [10] results showed the same effect to glycogen level in a liver of rats. Furthermore, according to results of this study, Cd exposure also led to increasing the glucose level in liver homogenate. The increased glucose level in a liver is consistent with some previously published reports. The result of Al Rikabi and Jawad [24] study showed that Cd exposure significantly increased the blood glucose. Sobha *et al.*, [25] also showed that Cd exposure could increased the liver, muscle, and blood glucose of *Catla catla*.

Glucose metabolism disruption by Cd in liver homogenate is mediated through several mechanisms. Cd has been widely reported to induce Free radical-induced oxidative stress causes membrane lipid

peroxidation, which may result in liver cell damage [26].

The Cd also damaged the liver cells by binding to -SH in mitochondria and secondary injury initiated by the activation of kupfer cells. Inactivation of -SH causes oxidative stress, mitochondrial permeability transition, and mitochondrial dysfunction. It is also suggested that kupfer cells release proinflammatory cytokines and chemokines which stimulate the migration and accumulation of neutrophils and monocytes in the liver. Several reports also suggested that liver cells damaged may be caused by ischemia due to sinusoidal endothelial cell dysfunction. Cd has been found to accumulate in endothelial cells leading to necrosis and denudation of hepatic sinusoids. Thus will disrupt the glucose metabolism [27].

In addition, the decreasing of glycogen level and increasing of glucose level in liver homogenate could affected the level of MG. It can see from the results of this present study. The MG level decrease with the presence of Cd. This might be due to liver cell damaged, resulting in the

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disruption of glucose metabolism pathway, as mentioned above. MG is produced from dihydroxyacetone phosphate, a side product of glycolysis, by the enzyme methylglyoxal synthase [28,29]. Liver cells damaged might be inhibited the glycolysis, and will decrease the MG level. It is well known that *gemor* have medicinal values. According to previous studies *gemor* have antiviral and anti-inflammation activity. Arifin *et al.*, [14] results, showed that the twig, leaves and barks of *gemor* contain the phytochemical constituents such as flavonoid, a phenolic compound, alkaloid, steroid, and triterpenoid. Those phytochemical constituents might be the reason the pharmacological activities of *gemor*.

In the present study, aqueous extracts of leaves and bark of *gemor* were evaluated for their effect on Cd-induced glucose metabolism alteration in liver homogenate. Of those two different parts of *gemor*, bark showed better activity to maintain the glycogen, glucose, and MG concentration under the influence of Cd.

The activity of leaves and bark of *gemor* may be caused by the phytochemical constituents in both parts of *gemor*. Results of Arifin *et al.*, showed that the bark and leaves of *gemor* contain flavonoid. Flavonoids are known to form complexes with heavy metals. It has been confirmed in numerous studies that flavonoids, function as antioxidants mainly by chelating metal ions. Flavonoids are hydroxylated phenolic substances and are known to be synthesized by plants in response to microbial infection. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [30]. Due to such properties, flavonoids appear to be a suitable antidote for heavy metal poisoning. In addition, the results also suggest bark extracts showed better activity than leaves extracts.

CONCLUSION

Thus, the present study concluded that the aqueous extract of bark and leaves of *gemor* showed significant activity to inhibited the glucose metabolism alteration by Cd in liver homogenate by using *in vitro* models. Also, the results of this present studies showed that bark are more effective than leaves of *gemor* to inhibited the glucose metabolism alteration in liver homogenate. Further studies are undergoing in order to clarify their molecular mechanisms.

ACKNOWLEDGEMENT

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CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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1. The name of the third author is Purwanto Budi Santosa and not just Purwanto
2. Punctuation has been corrected.
3. Description of figure were returned to earlier revision.
4. Equation has been changed using the equation tools.
5. The title in X and Y axis were changed to sentence case and bold.
6. Heading of figure also changed according to latest issues of IJPPS.

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AMELIORATIVE EFFECTS OF DIFFERENT PARTS OF *GEMOR* (*Nothaphoebe coriacea*) ON CADMIUM INDUCED GLUCOSE METABOLISM ALTERATION IN VITRO

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ABSTRACT

Objective: The present study was undertaken to investigate the ameliorative effect of the bark and leaves of *Nothaphoebe coriacea* (*gemor*) on Cadmium (Cd) induced glucose metabolism alteration in liver homogenate *in vitro*.

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Methods: Glucose metabolism alteration in liver homogenate was induced by the administration of Cadmium Sulphate ($CdSO_4$) at a dose 3 mg/l. Ameliorative effect of the leaves and bark extracts was determined by assessing the concentration of glycogen, glucose and Methylglyoxal (MG). Dubois hydrolytic method were used for liver glycogen and glucose concentration estimation. Dinitro Phenyl Hydrazine (DNPH) method was used for MG concentration estimation. For the present experiment, liver was taken from twelve healthy male adult rats (*Rattus norvegicus*). Liver samples then divided in four groups with: C served as control which contains liver homogenate only; T1 which contains liver homogenate+3 mg/L of $CdSO_4$; T2 which contains liver homogenate+3 mg/L of $CdSO_4$ +aqueous extracts of bark of *gemor*; and T3 which contains liver homogenate+3 mg/L of $CdSO_4$ +aqueous extracts of leaves of *gemor*. Solutions then incubated at 37°C for 1 hours and then was prepared for liver glycogen, glucose, and methylglyoxal (MG) concentration measurement.

Results: The results of this present studies showed that treatment with $CdSO_4$ significantly decreased the levels of glycogen and MG concentration, and increased the level of glucose in liver homogenate compared to control. The aqueous extracts of bark and leaves of *gemor* significantly increased the levels of glycogen and MG concentration, and decreased the level of glucose in liver homogenate compared to control. The aqueous extracts of the bark of *gemor* in comparison with $CdSO_4$ treatment group showed significant effect to maintain the glycogen, glucose, and MG concentration in liver homogenate. However, when compared to the aqueous extracts of leaves of *gemor* the result was not significant. The results suggest aqueous extracts of the bark of *gemor* was more effective to prevent the glucose metabolism alteration induced by $CdSO_4$ than the aqueous extracts of leaves of *gemor*.

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Conclusion: The present study demonstrated Cd could induced the glucose metabolism alteration in liver homogenate, and the aqueous extracts of bark and leaves of *gemor* showed the ameliorative effect to prevent this alteration. In addition, the bark was more effective than leaves of *gemor* to prevent the glucose metabolism alteration induced by Cd.

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Keywords: Cadmium, *Gemor*, Glucose Metabolism.

INTRODUCTION

In recent years, advancement in technology has led to high levels of industrialization leading to the discharge of effluents and emissions containing heavy metals into the environment [1]. Heavy metals are inorganic elements essential for plant growth in traces or very minute quantities. They are toxic and poisonous in relatively higher concentrations [2]. This toxic metals can enter the human body by the consumption of contaminated food crops, water or inhalation of dust [3]. Furthermore, this will lead serious health disorders [4-5]. Among these heavy metals, Cadmium (Cd) is one of the heavy metals that have great environmental health effects [6].

The Cd is widely distributed in the environment due to its use in many industries [7]. Cd are absorbed through ingestion by food and water, and inhalation [8]. Once absorbed, Cd irreversibly accumulates in the human body, in particularly in kidneys and other vital organs such the lungs or the liver [9]. One of the basic mechanisms of Cd toxicity is its negative influence on Cd enzymatic systems of cells, owing to the substitution of other metal ions in metalloenzymes and its very strong affinity to biological structures containing Sulphydryl groups (-SH), carboxyl and phosphate. Exposure to Cd metal is known to induce the formation of reactive oxygen species (ROS). This will lead to cellular damage and disturb many process, including glucose metabolism [10]. This will lead to a broad range of physiological, biochemical, and behavioral dysfunctions, including several metabolism pathway, such as glucose metabolism.

It has been found that Cd could change glycogen reserves and serum glucose levels by affecting the activities of liver enzymes that have a pivotal role in the glucose metabolism such as gluconeogenesis, glycogenesis, and glycolysis [11]. Results from Bashir *et al.*, [10] study showed that, Cd exposure could increased the levels of plasma glucose and decreased hepatic glycogen. Some investigations also showed that heavy metals could decrease the glycogen reserves in fish and invertebrates [12]. ~~To prevent this alteration, several compound might be useful, including parts of plants extracts. One of the plants that that can be used is *Nothaphoebe coriacea* (*gemor*)~~

Recently, there is global interest in non-synthetic, natural drugs derived from herbal sources due to better tolerance and minimum adverse drug reactions. *Nothaphoebe coriacea* (Family: *Lauraceae*) commonly known as "Gemor" *Gemor* is a vernacular or trade name of *gemor* bark producing tree species (or *gemor* tree), which belongs to genus *Nothaphoebe* of the family *Lauraceae*. The species has been commonly identified as belonging to the genus *Alseodaphne* in the same family. Locally they are called as *gemor*, *menuk* or *tempuloh*, which cover *Nothaphoebe coriacea* and *Nothaphoebe umbelliflora* [13]. According to Sosef *et al.*, *Nothaphoebe coriacea* occurs in Peninsular Malaysia, Singapore and Indonesia (Sumatra and Kalimantan); while *Nothaphoebe umbelliflora* occurs in Indo China, Thailand, Peninsular Malaysia, Singapore, Indonesia (Sumatra, Java, Kalimantan) and Papua New Guinea. In Indonesia, the two species are found naturally in swampy forests of Sumatra and Kalimantan [14]. is one such plant that *Gemor* tree was potentially have medicinal benefits of the parts of the tree are either

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leaves, twig, bark. According to the previous study, the bark of *gemor* known have viral activity for influenza and herpes virus [14]. Arifin *et al.*, [14] studies also showed that the different parts of *gemor* (twig, bark, and leaves) have anti-inflammation activity. It is also reported that the bark, twig, and leaves *gemor* contain a phytochemical components, such as alkaloid, steroid, flavonoid, saponin, triterpenoid, tannins, and phenolic compounds [15]. This might be the reason for all medicinal properties of different parts of gemor.

However, no data are available in the literature of bark and leaves of *gemor* on the alteration of glucose metabolism induced by Cd. Therefore we undertook the present investigation to examine the ameliorative effects of bark and leaves of *gemor* on glucose metabolism alteration induced by Cd through *in vitro* models.

MATERIAL AND METHODS

Collection and Identification of Plant Materials

The fresh leaves and bark of *gemor* was collected from Tumbang Nusa, Central Kalimantan, Indonesia. The plant was authenticated by Dr. Kade Sidiyasa, Technology Research Institute for Conservation of Natural Resources, Balikpapan, East Kalimantan . Before use, it was ensured that the leaves and bark were free from contamination, sand and no microbial growth. The bark and leaves were shade dried and was made into coarse powder using a commercial blender.

Preparation of Extracts

Extraction was done by decoction methods [16]. 100 g of shade-dried bark and leaves of *gemor* was weighed and 1000 ml of distilled water was added to it respectively. This mixture was taken in 1000 ml beaker and subjected to heating continuously for 30 minutes at a temperature of 90°C. Then

the mixture was allowed to cool on ~~to~~ room temperature and subjected to filtration by means of vacuum filter. The filtrate so obtained is concentrated, so that all the excess solvent is evaporated in order to get the concentrated extract.

Experimental Protocol

The liver samples were collected from 12 old male rats (*Rattus novergicus*) with 2-3 month old, weighing 200-250 gram. Then liver samples were taken by surgically procedure with ether as an anaesthesia. Then the liver fixed in phosphate buffer at pH 7.0. The liver was ground to form a liquid. Subsequently the solution was taken and centrifuged at 3500 rpm for 10 minutes and the top layer were taken and stored until it use.

Furthermore, the liver homogenate were prepared to experimental *in vitro* models. Samples divided into into 4 groups (1 control group and 3 treatment groups). Control (C) group: liver homogenate only, Treatment 1 (T1) group: liver homogenate + 3 mg/l~~l~~ of Cadmium ~~d~~Sulphate (CdSO₄)~~0~~₄, Treatment 2 (T2) group: liver homogenate + 3 mg/l~~l~~ of CdSO₄ + aqueous extracts of bark of *gemor*, Treatment 3 (T3) group: liver homogenate + 3 mg/l~~l~~ of CdSO₄ + aqueous extracts of leaves of *gemor*. Each solutions then incubated at 37° C for 1 hour. After incubation, liver glycogen, glucose, and M~~m~~methylglyoxal (MG) concentration were estimated.

All animals used and care were in compliance of the Ethics Commission of Faculty of Medicine, Unjversity of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia in 9 August 2015.

Estimation of Liver Glycogen Concentration

This assay was performed as described by Bidinotto *et al.*, [17]. Samples of liver were quickly separated from freeze~~d~~ tissues and transferred to essay tubes containing 1.0 ml of 6 n~~N~~ potassium

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hydroxide (KOH). The tubes were transferred to a boiling water bath and left along 3-5 minutes 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 sulphate (K₂SO₄) was appended. A cloudy white precipitate was formed and the supernatant was discharged after centrifuging at 3000 rpm for 3 minutes. It was added 2.5 ml of distilled water to the precipitate, which was promptly dissolved. Suitable aliquots from such solution were employed to Dubois reaction. Glycogen concentration is expressed in µmol of glucosil-glucose per gram of wet tissue.

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 minutes at 5000 rpm, the contents of glucose was 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's 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 (H₂SO₄) was added into one stroke developing strong heat of reaction. The product was determined at 540 nm in a single colorimeter [17].

Estimation of Liver Methylglyoxal Concentration

MG compounds are measured using modified Dinitro-Phenylhydrazine (DNPH) method. From each test solution, 0.5 ml solution were taken, and then each solution was divided to 2 tubes with 0.25 ml volume in each tube. The first tube was the sample (A) and the second tube was blank (B) solution. Then 1 ml DNPH were

added into each A tube and 1 ml HCl 2.5 molM into each B tube. The tubes were incubated for 45 minutes in room temperature and protected from light, then tubes were shaken with a vortex for 15 minutes. The next step is added 1 ml of trichloroacetic acid (TCA) 20% into each tube (A and B), then the tubes were incubated for 5 minutes. Tubes were centrifuged for 5 minutes with 1400 rpm of speed to separate the supernatant. The pellets are centrifuged and washed three times with the addition of 1 ml ethanol-ethyl acetate. The last step were added 1 ml of urea 9 molM and incubate the solution for 10 minutes in 37°C while it was shaken. The solution was centrifuged again for 5 minutes in 1400 rpm of speed. Then the absorbance was measured at λ = 390 nm (A1).

Furthermore, a total of 25 µL of the homogenate was added to 350 µL DNPH (0.1% DNPH in 2 N HCl) and then 2.125 ml distilled water was added. It is incubated for 15 minutes at 37°C, then 1.5 ml NaOH 10% was added. Absorbance was measured at λ = 576 nm (A2). MG level was calculated following to equation : MG level (%) = (A1/A2) x 100% [18].

RESULTS

Liver Glycogen Concentration

Figure 1 represented the mean values ± standard error (Mean ± SE) of liver glycogen concentration. Dispersion of measured values around each mean varied from 275.56 to 347.78. Control and Treatment with Cd (C and T1 group) led to higher-lower liver glycogen concentrations than treatment with bark or leaves of gemor (T2 and T3) other group of treatments. That data also suggest the highest liver glycogen concentration is was on C group and the lowest was the T1

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group. Statistical analysis test results showed that all groups of treatment significantly ($p < 0.05$) reduced liver glycogen concentration compared to control. Liver glycogen concentration was significantly higher in T2 group and lower

in T3 group in comparison with the T1 group. The data from figure 1 suggest that T2 group more effective to maintained the liver glycogen concentration than the T3 group.

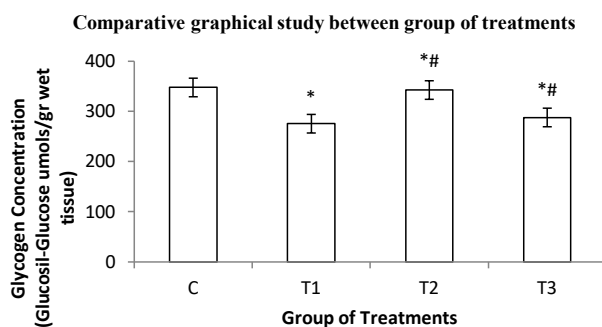


Figure 1. It shows the effect of cadmium and aqueous extracts of leaves and bark of *gemor* on liver glycogen concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment group. C represent control group; T1 represent Cd treatment group; T2 represent bark of *gemor* treatment group; and T3 represent leaves of *gemor* treatment group.

Liver Glucose Concentration

Figure 2 represented the mean values \pm standard error (Mean \pm SE) of liver glucose concentration. Dispersion of measured values around each mean varied from 137.00 to 399.45. All groups of treatment (T1, T2, and T3) showed a higher glucose concentration compared to control. Also, the data from figure 2 showed that treatment with Cd (T1) could increase the glucose concentration compared to control, while treatments with bark (T2) and leaves (T3) of *gemor* could

can decrease the glucose concentration compared to T1 group. Statistical analysis test results showed treatment with Cd, bark and leaves of *gemor* led to a significant increase of liver ~~their~~ glucose levels in comparison with the negative control group. Treatment with a bark of *gemor* led to significant decrease of the glucose concentration compared with Cd treatment group, and treatment with leaves of *gemor* also decrease the glucose concentration but non-statistically significant.

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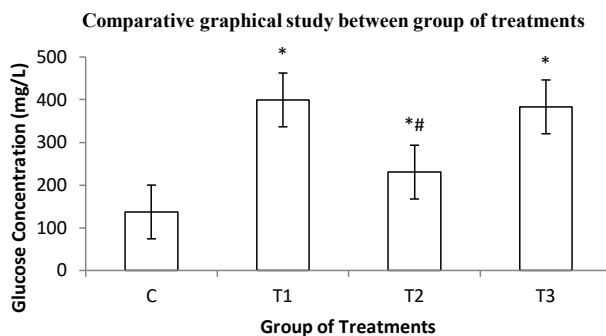


Figure 2. It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver glucose concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment group (T1). C represent control group; T1 represent Cd treatment group; T2 represent bark of *gemor* treatment group; and T3 represent leaves of *gemor* treatment group.

Liver Methylglyoxal Concentration

Figure 3 represented the mean values \pm standard error (Mean \pm SE) of liver MG concentration. Dispersion of measured values around each mean varied from 15.66 to 69.12. All groups of treatment (T1, T2, and T3) showed a lower MG concentration compared to control. Also, data from figure 3 showed that treatment with Cd (T1) could decrease the MG concentration compared to control, while treatments with bark (T2) and leaves (T3) of *gemor* cannot increase the MG concentration compared to T1 group. Statistical analysis test results showed treatment with Cd, bark and leaves of *gemor* led to a significant decrease of their levels in comparison with the control group. Treatment with a bark of *gemor* led to significant increase of the MG concentration compared with Cd treatment group, and treatment with leaves of *gemor* also increase the MG concentration but non-statistically significant.

DISCUSSION

The liver plays an astonishing array of vital functions in the maintenance, performance and regulating homeostasis of the body. It is involved with almost all the biochemical pathways to growth, a fight against the disease, nutrient supply, energy provision and reproduction. The major functions of the liver are a carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for the overall health and well-being [19].

The liver may be exposed to large concentrations of exogenous substances and their metabolites [20]. One of the exogenous substances that are harmful to the liver is heavy metal, such as Cd [21]. The Cd is one of the known environment toxins that is detrimental to liver function on exposure. This will lead to liver cell damage and will disrupt various metabolic pathways including glucose metabolism [22].

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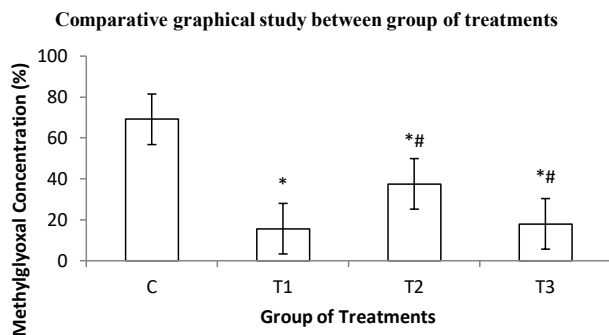


Figure 3. It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver MG concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment group (T1). C represent control group; T1 represent Cd treatment group; T2 represent bark of *gemor* treatment group; and T3 represent leaves of *gemor* treatment group.

Glucose metabolism alteration induced by Cd can be seen from the results of this present study. According to the result of this present studies, Cd exposure led to decrease glycogen concentration in liver homogenate. The results of our studies are supported by the other research. Bhati *et al.*, [23] results showed Cd could decrease the glycogen level in a liver of rats. Also, Bashir *et al.*, [10] results showed the same effect to glycogen level in a liver of rats. Furthermore, according to results of this study, Cd exposure also led to increase the glucose level in liver homogenate. The increased glucose level in a liver is consistent with some previously published reports. The result of Al Rikabi and Jawad [24] study showed that Cd exposure significantly increased the blood glucose. Sobha *et al.*, [25] also showed that Cd exposure could increased the liver, muscle, and blood glucose of *Catla catla*.

Glucose metabolism disruption by Cd in liver homogenate is mediated through several mechanisms. Glucose metabolism alteration by Cd has been suggested

through several mechanism. Cd has been widely reported to induce Free radical-induced oxidative stress causes membrane lipid peroxidation, which may result in liver cell damage [26].

The Cd also damaged the liver cells by binding to sulfhydryl groups in mitochondria and secondary injury initiated by the activation of kupffer cells. Inactivation of sulfhydryl groups causes oxidative stress, mitochondrial permeability transition, and mitochondrial dysfunction. It is also suggested that kupffer cells release proinflammatory cytokines and chemokines which stimulate the migration and accumulation of neutrophils and monocytes in the liver. Several reports also suggested that liver cells damaged may be caused by ischemia due to sinusoidal endothelial cell dysfunction. Cd has been found to accumulate in endothelial cells leading to necrosis and denudation of hepatic sinusoids. Thus will disrupt the glucose metabolism [27].

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In addition, the decreasing of glycogen level and increasing of glucose level in liver homogenate could affected the level of MG. It can seen from the results of this present study. The MG level decrease with the presence of Cd. This might be due to liver cell damaged, resulting in the disruption of glucose metabolism pathway, as mentioned above. MG is produced from dihydroxyacetone phosphate, a side product of glycolysis, by the enzyme methylglyoxal synthase [28,29]. Liver cells damaged might be inhibited the glycolysis, and will decrease the MG level.

It is well known that *Nothaphoebe eoriaceae* (*gemor*) have a medicinal values. According to previous studies *gemor* have antiviral and anti-inflammation activity. Arifin *et al.*, [14] results, showed that the twig, leaves and barks of *gemor* contain the phytochemical constituents such as; flavonoid, a phenolic compound, alkaloid, steroid, and triterpenoid. Those phytochemical constituents might be the reason the pharmacological activities of *gemor*.

In the present study, aqueous extracts of leaves and bark of *gemor* were evaluated for their effect on Cd-induced glucose metabolism alteration in liver homogenate. Of those two different parts of *gemor*, bark showed better activity to maintain the glycogen, glucose, and MG concentration under the influence of Cd.

The activity of leaves and bark of *gemor* may be caused by the phytochemical constituents in both parts of *gemor*. Results of Arifin *et al.*, showed that the bark and leaves of *gemor* contain flavonoid. Flavonoids are known to form complexes with heavy metals. It has been confirmed in numerous studies that flavonoids, function as antioxidants mainly by chelating metal ions. Flavonoids are hydroxylated phenolic substances and are

known to be synthesized by plants in response to microbial infection. Functional hydroxyl groups in flavonoids mediate their antioxidant effects by scavenging free radicals and/or by chelating metal ions [30]. Due to such properties, flavonoids appear to be a suitable antidote for heavy metal poisoning. In addition, the results also suggest bark extracts showed better activity than leaves extracts.

CONCLUSION

Thus, the present study concluded that the aqueous extract of bark and leaves of *gemor* showed significant activity to inhibited the glucose metabolism alteration by Cd in liver homogenate by using *in vitro* models. Also, the results of this present studies showed that bark are more effective than leaves of *gemor* to inhibited the glucose metabolism alteration in liver homogenate. Further studies are undergoing in order to clarify their molecular mechanisms.

ACKNOWLEDGEMENT

The authors acknowledge with thanks the support of the Banjarbaru Forestry Research Institute, Banjarbaru under the contract No. 5935.001.014 Mak DIPA BPK Banjarbaru.

CONFLICT OF INTEREST

We declare that we have no conflict of interest.

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Author Response to the comments of reviewers :

1. The format of the article has been corrected in accordance with the format of the journal.
2. The author has reduced and added a few sentences in the introduction to justify rationality of behind selection of the plant for presented study.
3. Authentication of the plant was added in material and methods sub-chapter collection and identification of plant materials.

4. Ethical permission of animal studies was added in material and methods sub-chapter experimental protocol.
5. Abbreviation in the abstract and the text has been changed in accordance with reviewer comments.
6. Abstract headings has been bold.
7. Symbol and units has been changed according to International System of Units (SI).
8. Unit degree celcius has been changed to °C.
9. Error bars for figure 1-3 has been included in the figures.
10. Grammatical and punctuation errors has been changed using grammarly.
11. Heading of figures has been rectified.
12. References has been corrected. There is no number with volume.
13. Pagination style also has been corrected according to the latest article in IJPPS.

GEMOR (Nothaphoebe coriacea) ON CADMIUM INDUCED GLUCOSE METABOLISM ALTERATION IN VITRO" that it has been recommended for publication after peer review.

I acknowledge you receipt of registration fee by NEFT for IJPPS 8158.

Your article is now accepted for publication and your article is scheduled to be published in Vol 7 Issue 11, November 2015.

Important Announcement:

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