The Protective Efficacy of Kelakai (*Stenochlaena Palustris*) on Cadmium-induced Glucose Metabolism Alteration In Vitro

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Keywords: Cadmium, Glucose Metabolism, Stenochlaena palustris

Abstract: The objectives of this study were to determine the protective effect of kelakai (*Stenochlaena palustris*) leaves extract on cadmium (Cd)-induced glucose metabolism alteration in vitro. The protective effect of plant extracts extract was determined by assessing the activity of pancreas amylase and liver glucokinase, the concentration of liver glucose, glycogen, and methylglyoxal (MG). In this present study, the liver and pancreas samples were obtained from 32 old male *Rattus novergicus*. Each model then divided into 4 groups consisting of: pancreas or liver + 0.3 mg/l $CdSO_4$ (T1); pancreas or liver + 0.3 mg/l $CdSO_4$ + 5 mg/l leaves extract (T2); pancreas or liver + 0.3 mg/l $CdSO_4$ + 10 mg/l leaves extract (T3); and pancreas or liver + 0.3 mg/l $CdSO_4$ + 15 mg/l leaves extract (T4). Results of this present study shows that the administration of *S. palustris* leaves extract could significantly decreased the pancreas amylase activity, the level of liver glucose and MG, and significantly increase the liver glucokinase activity, and the level of liver glycogen ($P < 0.05$). In conclusions, the results of this present study indicated that the administration of *S. palustris* leaves extract could improve the glucose metabolism alteration by Cd.

1 INTRODUCTION

Industrialization and urbanization development have resulted in increasing of environment contamination by several toxic substances, including heavy metal (Anyakora et al., 2013). Among heavy metals, cadmium (Cd) is one of the heavy metal that is very toxic to human body even at low concentration (Rahman et al., 2014). If Cd enters the human body, it could irreversibely accumulates and affect some vitals organs such as liver, pancreas, kidney, and nervous system (Suhartono et al., 2015a; Suhartono et al., 2016; Khorasgani et al., 2013). Also, Cd could affect some metabolism pathway including glucose metabolism (Suhartono et al., 2015b).

It has been found that Cd could decreased the glycogen reserves and increased the glucose levels in liver homogenate (Suhartono et al., 2015b). Also, Bashir et al. (2014) study have shown that Cd could increased the level of plasma glucose and decreased the level of liver glycogen. Several investigators have confirmed that Cd could affect the glucose metabolism via several pathway. Cd altered activites of carbohydrate metabolizing enzymes, including hexokinase, glucokinase, phosphofructokinase and amylase (Navaneethan et al., 2014; Bajo et al., 2014; Slencu et al., 2014). Also, Cd could induced the liver and pancreas cells damaged resulted in glucose metabolism alteration (Khorasgani et al., 2014; Suhartono et al., 2015b).

It has been long appreciated that Cd toxicity could prevent by a number of natural antioxidants.

Suhartono, E., Oktaviani, I., Adenan, . and Thalib, I.
The Protective Efficacy of Kelakai (Stenochlaena Palustris) on Cadmium-induced Glucose Metabolism Alteration In Vitro.
DOI: 10.5220/0008788101560162
In *Proceedings of the 2nd Syiah Kuala International Conference on Medicine and Health Sciences (SKIC-MHS 2018)*, pages 156-162
ISBN: 978-989-758-438-1
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In particular, kelakai (*S. palustris*), a local plants that growth in Indonesia, especially South Kalimantan known to be rich source of antioxidants. Our previous reports shows that *S. palustris* contains flavonoid and possess several antioxidants activities (Suhartono et al., 2012). Also, another previous study suggest that the *S. palustris* extracts can slow down the formation of MG, AOPPs, and PC in bovine serum albumin in vitro (Suhartono et al., 2016).

In view of the antioxidant properties of *S. palustris*, it is noteworthy to consider that *S. palustris* might bring out beneficial effects on Cd-induced glucose metabolism alteration in vitro. Therefore, the present study has been designed to evaluate the protective efficacy of *S. palustris* on Cd-induced glucose metabolism alteration in vitro.

2 MATERIAL AND METHODS

2.1 Collection and Extraction of Plant Materials

The fresh leaves of *S. palustris* were obtained from Gambut subdistrict, South Kalimantan, Indonesia in February 2016 and identified by Department of Biology, Pharmacy Study Program, Faculty of Mathematics and Natural Sciences, Lambung Mangkurat University, South Kalimantan, Indonesia. The plant parts were separated, shade dried and powdered. Then, Powdered material of *S. palustris* leaves is taken for maceration with 150 ml of distilled water for 1 hr on rotary shaker. The extract then filtered using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath (Suhartono et al., 2012).

2.2 Experimental Section

The liver and pancreas samples were obtained from 32 old male rats (*Rattus novergicus*) with 2-3-month-old, weighing 200-250 g. The liver and pancreas samples were taken surgically with ketamine as anaesthesia. Experiments performed complied with the rulings of the Institute of Laboratory Animal Resources, Commission on Life Sciences, National Research Council and were approved by the Ethical Committee of the Faculty of Medicine, University of Lambung Mangkurat, Banjarbaru, South Kalimantan, Indonesia.

The liver and pancreas samples then fixed in phosphate buffer solutions at pH 7.0. The liver and pancreas samples for analysis were homogenized, respectively. The homogenates were centrifuged at 3500 rpm for 10 min and the top layer was taken and stored until it uses.

Liver and pancreas samples were divided into 4 groups with 6 samples of in each group. Group 1 (T1): liver or pancreas homogenates + 0.3 mg/l cadmium sulphate ($CdSO_4$); group 2 (T2): liver or pancreas homogenates + 0.3 mg/l $CdSO_4$ + 5 mg/l of aqueous extracts of *S. palustris*; group 3 (T3): liver or pancreas homogenates + 0.3 mg/l $CdSO_4$ + 10 mg/l of aqueous extracts of *S. palustris*; group 4 (T4): liver or pancreas homogenates + 0.3 mg/l $CdSO_4$ + 15 mg/l of aqueous extracts of *S. palustris*. Each solution then incubated at 37°C for 1 hour. After incubation, pancreas amylase activity, liver glucokinase activity, liver glycogen, glucose, and MG concentration was estimated.

2.3 Pancreas Amylase Activity Analysis

The pancreas amylase activity was measured according to the Smith and Roe method (Smith and Roe, 1949). Pipette 5 ml of 1.2% starch solution (60 mg) at approximately 90ºC, 3 ml of phosphate buffer, and 1 ml of 0.5 M sodium chloride into each of two test-tubes. Into a third tube (C), the blank, pipette 5 ml of distilled water, 3 ml of phosphate buffer, and 1 ml of 0.5 M sodium chloride. Place all tubes in a water bath at 37oC until they have reached the temperature of the water bath. To Tube A, add 1 ml of enzyme solution. Keep all tubes in the water bath for exactly 30 minutes. Promptly add 2 ml of N-hydrochloric acid to each tube. This brings the pH below 2, a step that stops amylase action in the digest tube and prevents action of the enzyme next added to the control tube. Add 1 ml of enzyme solution to tubes B (control) and C (blank) and mix thoroughly. Pipette 2 ml of each of these reaction mixtures into appropriately labelled 500 ml volumetric flasks containing about 400 ml of distilled water and 5 ml of N-hydrochloric acid. Add 1 ml of iodine reagent to each flask and make up to volume. The resulting blue solutions are decanted into cuvettes and read in a photoelectric calorimeter at a wave-length of 620 mp. Solution from Tube B gives the iodine colour value without amylase action and solution from Tube A gives the value after enzyme action.

Calculations – Let D = 2 – log G = Optical Density

$$\frac{\text{(D of Control)} - \text{(D of Digest)}}{\text{(D of Control)}} \times 60 = \text{mg of starch hydrolysed}$$

The amylase unit is defined as the amount of enzyme that under the conditions of this procedure, with 60 mg. of starch present, will hydrolysed 10 mg. of starch in 30 minutes to a stage at which no colour is given with iodine at 620 rnp. The definition of this amylase unit was established to make the unit conform as closely as possible to the units of methods in general use.

2.4 Liver Glucokinase Activity Analysis

Glucose concentration (100 mM, 200 mM, 300 mM, 400 mM and 500 mM), each concentration was taken added 3 ml and 3 ml of phosphate buffer pH 7. Furthermore, mixed until homogeneous. A total of 1 ml homogenate is added to each mixture, and then measured as the levels of glucose [G0]. After 20 minutes, each mixture of glucose is measured again [G1] by the method of hydrolytic Duboie's. The rate of oxidation of glucose by glucokinase (v) is expressed in changes in the concentration of glucose per minute (Bustos and Iglesias, 2000).

2.5 Liver Glycogen Level Analysis

This assay was performed as described by Bidinotto et al. (Bidinotto et al., 1997) Samples of liver were quickly separated from freeze tissues and transferred to essay 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.

2.6 Liver Glucose Level Analysis

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 (Bidinotto et al., 1997).

2.7 Liver MG Level Analysis

MG compounds are measured using modified Dinitro-Phenyl hydrazine (DNPH) method (Suhartono et al., 2014). From each test solution, 0.5 ml solution was 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, and 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 was added 1 ml of urea 9 mol/l and incubates 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 λ = 390 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 λ = 576 nm (A1).

MG level was calculated following to equation:

$$\text{MG Level (\%)} = \frac{A1}{\Delta A} \times 100\%$$

2.8 Statistical Analysis

The results were expressed as mean ± SE for six replicates. Significance of mean differences of all parameters between group of treatments were

statistically compared using Kruskal-Wallis test and followed by Mann-Whitney test for multiple range test. Significance was set at P<0.05. The software used for the data analysis were the Statistical Package for the Social Sciences (SPSS) version 16.0 and Microsoft Excel 2010 for Windows Vista.

3 RESULTS

This present study which was undertaken to assess the protective effects of *S. palustris* on Cd-induced glucose metabolism alteration in in vitro condition. Several parameters were measured such as, amylase pancreas, liver glucose, glycogen, and MG level, and liver glucokinase activity.

Figure 1 shows the mean comparison for pancreas amylase activities between group of treatments. The activity of pancreas amylase is higher in T1 group than all group of treatments. Treatment with *S. palustris* led to lower pancreas amylase activity, with the lowest activity is in T4 group. Kruskal-Wallis test results shows that all groups of treatment are significantly (p<0.05) different. Then, we used Mann-Whitney test for multiple comparison test. b, c, d, and e indicate that significant differences compared with T1, T2, T3, and T4 group, respectively. Mann-Whitney test results show that there is a significant difference between a group of treatments except between T1-T2.

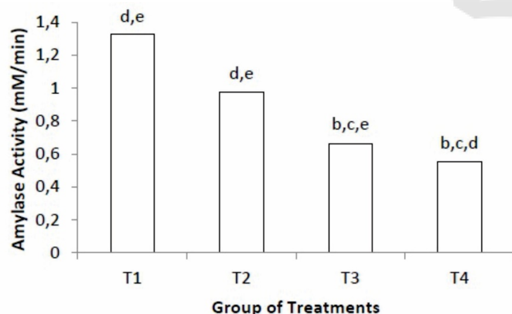

Figure 1: Comparison of pancreas amylase activity between group of treatments. Values are mean ± SEM of six replicates in each group treatments. b: Significantly different when compared to Group T1; c: Significantly different when compared to Group T2. d: Significantly different when compared to Group T3; and e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and followed by Mann-Whitney U test (P<0,05).

Figure 2 shows the mean comparison for liver glucokinase activities between group of treatments. The activity of liver glucokinase were lower in T1 group compared with the all group of treatments. Also, the result shows that the activity of liver glucokinase seems higher, with the highest activity is in group T4. Kruskal-Wallis test results shows that all groups of treatment are significantly (p<0.05) different. Also, b, c, d, and e indicate the significant differences compared to T1, T2, T3, and T4 group, respectively. Mann-Whitney test results show that there is a significant difference between a group of treatments except between T2-T3.

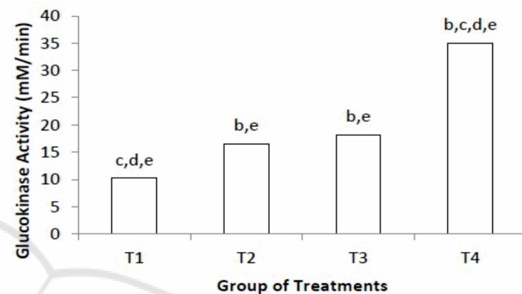

Figure 2: Comparison of liver glucokinase activity between group of treatments. Values are mean ± SEM of six replicates in each group treatments. b: Significantly different when compared to Group T1; c: Significantly different when compared to Group T2. d: Significantly different when compared to Group T3; and e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and followed by Mann-Whitney U test (P<0,05).

Figure 3 represented the mean values ± standard error (mean ± SEM) of liver glucose concentration. Dispersion of measured values around each mean varied from 23.661 to 42.883 mM. The data from figure 3 shows that T1 group have a higher liver glucose concentration than another group of treatments, while T2-T4 group have a lower liver glucose concentration than T1 group. Kruskal-Wallis test results shows that all groups of treatment are significantly (p<0.05) different. The letters indicate the multiple comparison using Mann-Whitney test as mentioned above. Mann-Whitney test results show that there is a significant difference between a group of treatments except between T1-T2, and T2-T3.

Figure 4 represented the mean values ± standard error (mean ± SEM) of liver glycogen concentration. Dispersion of measured values around each mean varied from 0.600 to 2.599 glucosil-glucose µmol/gr

wet tissue. The data from figure 4 shows that T1 group have a lower liver glycogen concentration than another group of treatments, while T2-T4 group have a higher liver glycogen concentration than T1 group. Kruskal-Wallis test results shows that all groups of treatment are significantly ($p<0.05$) different. Also, the letters indicate Mann-Whitney test results. Mann-Whitney test results show that there is a significant difference between a group of treatments except between T1-T2, and T3-T4.

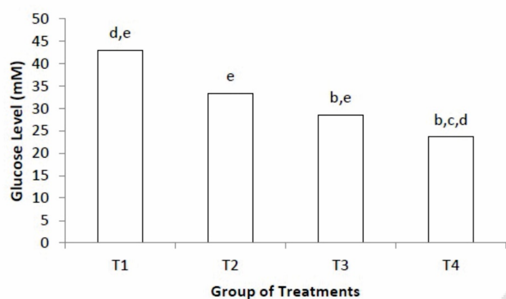

Figure 3: Comparison of liver glucose level between group of treatments. Values are mean ± SEM of six replicates in each group treatments. b: Significantly different when compared to Group T1; c: Significantly different when compared to Group T2. d: Significantly different when compared to Group T3; and e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and followed by Mann-Whitney U test (P<0,05).

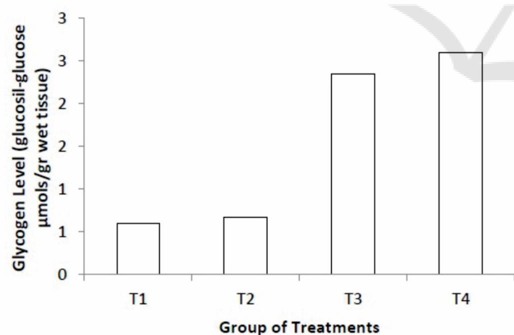

Figure 4: Comparison of liver glycogen level between group of treatments. Values are mean ± SEM of six replicates in each group treatments. b: Significantly different when compared to Group T1; c: Significantly different when compared to Group T2. d: Significantly different when compared to Group T3; and e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and followed by Mann-Whitney U test (P<0,05).

Figure 5 shows the mean comparison for liver MG level between group of treatments. The level of MG was higher in T1 group than another group of treatments. Also, the result shows that the level of liver MG seems lower, with the lowest level are in group T3 and T4. Kruskal-Wallis test results shows that all groups of treatment are significantly ($p<0.05$) different. The letters indicate Mann-Whitney test results. Mann-Whitney test results show that there is a significant difference between a group of treatments except between T1-T2, and T3-T4.

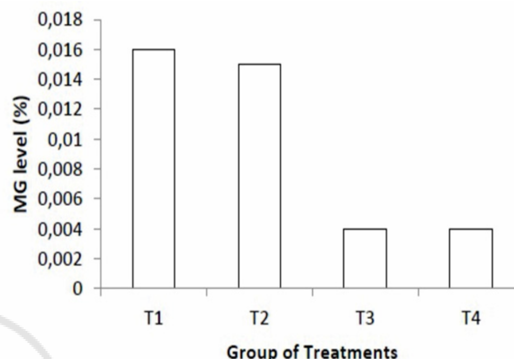

Figure 5: Comparison of liver MG level between group of treatments. Values are mean ± SEM of six replicates in each group treatments. b: Significantly different when compared to Group T1; c: Significantly different when compared to Group T2. d: Significantly different when compared to Group T3; and e: Significantly different when compared to Group T4. Comparison of variables between the groups was performed with Kruskal-Wallis test and followed by Mann-Whitney U test (P<0,05).

4 DISCUSSION

It is widely known that the pancreas and liver play an important role in glucose metabolism (Pap, 2004; Kalsbeek et al., 2014). Pancreas secrete hormones such as insulin and glucagon, and several enzymes such as, amylase (Pap, 2004), while liver balancing glucose entry into and out of the circulation (Kalsbeek et al., 2014; Bechmann et al., 2012). Thus, the effect of protective agents on tissues such as the liver and pancreas that regulate glucose metabolism is an interesting area to explore.

In this present study, we investigate the protective effect of *S. palustris* on Cd-induced glucose metabolism in vitro by measuring several parameters such as pancreas amylase activity, liver glucokinase activity, liver glucose, glycogen, and MG level. From the result, in can be seen that Cd exposure increase amylase activity. The results of our studies are supported by the other research by

Khorasgani et al. (2013). Results of that study showed that Cd could increase the amylase activity level in a pancreas of rats. Also, El-Aziz and El-Mottaleb (2015) results showed the same effect but in amylase activity in a serum of rats. The increasing of amylase activity has known as a result from pancreatitis or from damage amylase secretory cells by Cd (Abedi et al., 2013). Result of this present study also suggested that *S. palustris* extract shows a protective effect especially in higher concentration. This protective effect might be the presence of phenolic compounds in *S. palustris* extracts. These results are consistent with our previous report which found that the *S. palustris* extracts comprise flavonoid and possess some antioxidant activity (Suhartono et al., 2012). This could inhibit the pancreatitis and chelate some metal including Cd to improve the amylase activity.

Results of this present study also indicated that Cd exposure led to increase the glucose level and decrease the glycogen level in a liver of rats. These results are consistent with several previous reports (Suhartono et al., 2015b; Bashir et al., 2014; Bhati et al., 2014; Al Rikabi and Jawad, 2013; Sobha et al., 2007). According to several previous reports, Cd could induce the liver cells damage by interrupt the pro- and anti-oxidant balance (Matovic et al., 2011; Skipper et al., 2016; Suhartono et al., 2013). The result of this present study indicated that *S. palustris* extract could improve the level of glucose and glycogen in liver cells homogenate, again better protective effect in greater kelakai concentration. This result was similar with our previous reports but with bark and leave of *Nothaphoebe coriacea* extract (Suhartono et al., 2016). The reason why *S. palustris* have a protective effect to inhibited the Cd-induced glucose metabolism alteration may be the same reason as mentioned in the previous paragraph in this section.

According to the result of this present study, Cd exposure could affect the MG level in liver cells homogenate. These results contrast with our previous report. Our previous report show that Cd could decrease the MG level in liver cells homogenate (Suhartono et al., 2015). However, in another several previous reports Cd could induced the formation of MG both in in vitro and in vivo condition, but in another organs, such as kidney (Suhartono et al., 2016; Suhartono et al., 2014; Husna et al., 2014). It is well known that MG is the precursor of quantitatively important advanced glycation end products (AGEs) (Rabbani and Thornalley, 2014). MG is form via several mechanism, including auto-oxidation of glucose, which leads to glyoxal formation, decomposition of amadori products (3-deoxyglucosone) and fragmentation of glycerinaldehyde-3-phosphate and dihydroxyacetone phosphate during glycolysis (Jorgens et al., 2015).

5 CONCLUSIONS

S. palustris extracts possesses a protective effect against Cd-induced glucose metabolism alteration in vitro. The protective effects might be some phytochemical constituents contained in *S. palustris* extracts. Further studies will be worthwhile to explore the exact phytochemicals constituents in *S. palustris* extract and molecular protective effect mechanism *S. palustris* extract.

REFERENCES

Abedi Z, Hasantabar F, Khalesi MK, Babaei S. 2013. Effect of sublethal concentrations of cadmium, lead and chromium on some enzymatic activities of common carp; *Cyprinus carpio*. World Journal of Zoology, 8 (1), pp. 98-105.

Al Rikabi AA and Jawad AADH. 2013. Protective effect of ethanolic ginger extract against cadmium toxicity in male rabbits. Basrah Journal of Veterinary Research, 12, pp. 13-29.

Anyakora C, Ehianeta T, Umukoro O. 2013. Heavy metal levels in soil samples from highly industrialized Lagos environment. African Journal of Environmental Science and Technology, 7 (9), pp. 917-924.

Bajo MJR, Atauri P, Ortega F, Westerhoff HV, Gelpi JL, Centelles JJ, Cascante M. 2014. Effects of cadmium and mercury on the upper part of skeletal muscle glycolysis in mice. PLoS One, 9(1), e80018. DOI: 10.1371/journal.pone.0080018.

Bashir N, Manoharan V, Prabu SM. 2014. Ameliorative effects of grape seed proanthocyanins on cadmium induced metabolic alterations in rats. International Journal of Biological Research, 2, pp. 28-34.

Bechmann LP, Hannivoort RA, Gerken G, Hotamisligil GS, Trauner M, Canbay, A. 2012. The interaction of hepatic lipid and glucose metabolism in liver diseases. Journal of Hepatology, 56, pp. 952–964.

Bhati Sl, Ranga D, Meena DC, Agarwal M, Chakrawarti A, Purohit RK. 2014. Ameliorative effect of Emblica officinalis in mice liver. World Journal of Pharmaceutical Research, 3, pp. 846-863.

Bidinotto PM, Moraes G, Souza RHS. 1997. Hepatic glycogen and glucose in eight tropical fresh water teleost fish: a procedure for field determinations of micro samples. B Tec Cepta Pirassununga, 10, pp. 53-60.

Bustos DM and Iglesias AA. 2000. The kinetic properties of liver glucokinase and its function in glucose physiology as a model for the comprehensive study of enzymes' kinetic parameters and reversible inhibitors. Biochemistry and Molecular Biology, 28 (6), pp. 332-337.

El-Aziz EA and El-Mottaleb NAA. 2015. The potential protective effects of tetrahydrobiopterin on cadmium-induced pancreatic changes in male rats. Bulletin Egypt Social Physiological Science, 35 (3), pp. 62-74.

Husna AH, Ramadhani EA, Eva DT, Yulita AF, Suhartono E. 2014. The role formation of methylglyoxal, carbonyl compound, hydrogen peroxide and advance oxidation protein product induced cadmium in ovarian rat. International Journal of Chemical Engineering and Application, 5 (4), pp. 319-323.

Jorgens, K, Stoll SJ, Pohl J, Fleming TH, Sticht C, Nawroth PP, Hammes HP, Kroll J. 2015. High tissue glucose alters intersomitic blood vessels in zebrafish via methylglyoxal targeting the VEGF receptor signalling cascade. Diabetes, 64, pp. 213-225.

Kalsbeek, Andries, Fleur S, Fliers E. 2014. Circadian control of glucose metabolism. Molecular Metabolism, 3(4), pp. 372–383.

Khorasgani EM, Haghdoost IS, Sedaghat R, Mortazavi P, Roghani M. 2013. *Satureja hortensis* L. alcoholic extract ameliorates cadmium-induced pancreatic damage in rats. Middle-East Journal of Science Research, 15 (1), pp. 32-35.

Matovic V, Buha A, Bulat Z, Dukic-Cosic D. 2011. Cadmium toxicity revisited: focus on oxidative stress induction and interactions with zinc and magnesium. Arhiv Hig Rada Toksikol, 62(1), pp. 65-75.

Navaneethan D and Rasool MK. 2014. An experimental study to investigate the impact of p-coumaric acid, a common dietary polyphenol, on cadmium chloride-induced renal toxicity. Food Function, DOI: 10.1039/c4fo00346b.

Pap A. 2004. Effects of insulin and glucose metabolism on pancreatic exocrine function. International Journal of Diabetic Metabolism, 12, pp. 30-34.

Rabbani N and Thornalley PJ. 2014. The critical role of methylglyoxal and glyoxalase 1 in diabetic nephropathy. Diabetes, 63, pp. 50-52.

Rahman M, Gul S, Ajmal M, Iqbal A, Achakzai A. 2014. Removal of cadmium from aqueous solutions using excised leaves of quetta pine (*Pinus halepensis mill.*). Bangladesh Journal of Botany, 43 (3), pp. 277-281.

Skipper A, Sims JN, Yedjou CG, Tchounwou PB. 2016. Cadmium chloride induces DNA damage and apoptosis of human liver carcinoma cells via oxidative stress. International Journal of Environmental Research and Public Health, 13 (88), pp. 1-10.

Slencu BG, Ciobancu C, Solcan C, Anton A, Ciobanu St, Solcan Gh, Cuciureanu R. 2014. Effect of selenium supplementation on serum amylase, lactate dehydrogenase and alkaline phosphatase activities in rats exposed to cadmium or lead. Agronomical Research in Moldavia, XLVII (4), pp. 160.

Smith BW and Roe JH. 1949. Photometric method for the determination of α-amylase in blood and urine, with use of starch-iodine colour. Journal of Biological Chemistry, 179, pp. 53-59.

Sobha K, Poornima A, Harini P, Veeraiah K. 2007. A study on biochemical changes in the fresh water Fish, Catla catla (Hamilton) exposed to the heavy metal toxicant cadmium chloride. Kathmandu University Journal of Sciences Engineering and Technology, I, pp. 1-11.

Suhartono E, Viani E, Rahmadhan MA, Gultom IS, Rakhman MF, Indrawardhana D. 2012. Screening of medicinal plant for total flavonoid and antioxidant activity in South Kalimantan of Indonesian. International Journal of Chemical Engineering and Applications, 3 (4), pp. 297-299.

Suhartono E, Triawanti, Yunanto A, Firdaus RT, Iskandar. 2013. Chronic cadmium hepatooxidative in rats: treatment with haruan fish (*Channa striata*) extract. APCBEE Procedia, 5, pp. 441-445.

Suhartono E, Triawanti, Leksono AS, Djati MS. 2014. The role of cadmium in protein glycation by glucose: Formation of methylglyoxal and hydrogen peroxide in vitro. Journal of Medical and Biological Engineering, 3, pp. 59-62.

Suhartono E, Iskandar, Hamidah S, Arifin YF. 2015a. Phytochemical constituents' analysis and neuroprotective effect of leaves of gemor (*Nothaphoebe Coriacea*) on cadmium-induced neurotoxicity in rats: An in-vitro study. International Journal of Toxicological and Pharmacological Research, 7(6), pp. 297-302.

Suhartono E, Santosa PB, Iskandar. 2015b. Ameliorative effects of different parts of gemor (*Nothaphoebe coriacea*) on cadmium induced glucose metabolism alteration in vitro. International Journal of Pharmaceutical and Pharmacological Science, 7 (11), pp. 1-4.

Suhartono E, Bahriansyah M, Triawanti. 2016. The inhibition effect of kelakai (*Stenochlaena palustris*) extract on cadmium-induced glycation and fructation in-vitro. International Journal of Pharmaceutical and Clinical Research, 8 (4), pp. 248-253.