

Original Article

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 was 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 the 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]. 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 sulphhydryl groups (-SH), carboxyl and phosphate [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].

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 have medicinal benefits of the parts of the tree are either 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.

MATERIALS 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 were 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 beakers and subjected to heating continuously for 30 min at a temperature of 90 °C. Then the mixture was allowed to cool on room temperature and subjected to filtration by means of the 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 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 was taken and stored until it uses.

Furthermore, the liver homogenate were prepared to experimental *in vitro* models. Samples divided 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 (CdSO₄), Treatment 2 (T2) group: liver homogenate+3 mg/l of CdSO₄+aqueous extracts of bark of *gemor*, Treatment 3 (T3) group: liver homogenate+3 mg/l of CdSO₄+aqueous extracts of leaves of *gemor*. Each solution then incubated at 37 °C for 1 hour. After incubation, liver glycogen, glucose, and Methylglyoxal (MG) concentration was estimated.

All animals used and care was in compliance of the Ethics Commission of the 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 (K₂SO₄) 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 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 (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-Phenyl hydrazine (DNPH) method [18]. 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 $\lambda = 390$ 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 $\lambda = 576$ nm (A1).

MG level was calculated following to equation:

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

RESULTS

Liver glycogen concentration

Fig. 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. Treatment with Cd (T1 group) led to lower liver glycogen concentrations than other group of treatments. That data also suggests 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 fig. 1 suggest that T2 group more effective to maintain the liver glycogen concentration than the T3 group.

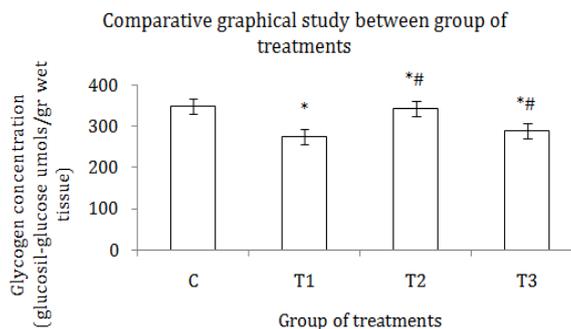


Fig. 1: It shows the effect of cadmium and aqueous extracts of leaves and bark of *gemor* on liver glycogen concentration. Values are mean±SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment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

Liver glucose concentration

Fig. 2 represented the mean values±standard error (mean±SEM) 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 fig. 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 decreases the glucose concentration but non-statistically significant.

Liver methylglyoxal concentration

Fig. 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 fig. 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 increases the MG concentration but non-statistically significant.

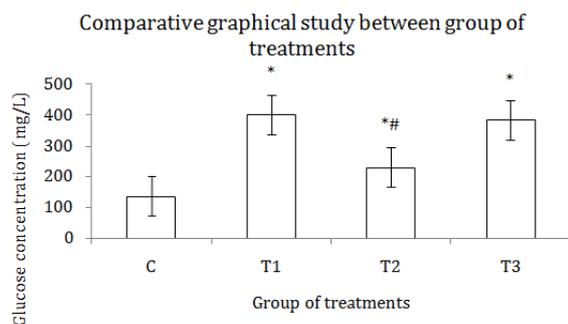


Fig. 2: It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver glucose concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment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

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].

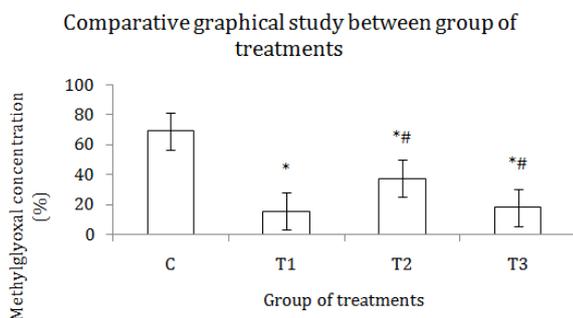


Fig. 3: It shows the effect of Cd and aqueous extracts of leaves and bark of *gemor* on liver MG concentration. Values are mean \pm SEM of four replicates in each groups of treatment. Statistical significance * $p < 0,05$ in comparison with control group, # $p < 0,05$ in comparison with Cd treatment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

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 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* has 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 inhibit 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.

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CONFLICT OF INTERESTS

We declare that we have no conflict of interest.

REFERENCES

1. Olukanni DO, Adeoye DO. Heavy metal concentrations in road side soils from selected locations in the lagos metropolis, Nigeria. *Int J Eng Technol* 2012;2:1743-52.
2. Al Obaidy AHM, Al Mashhady AAM, Awad ES, Kadhem AJ. Heavy metals pollution in surface water of mahrut river, Diyala, Iraq. *Int J Adv Res* 2014. 2:1039-44.
3. Mahmood A, Maleek RN. Human health risk assessment of heavy metals via consumption of contaminated vegetables collected from different irrigation sources in lahore, Pakistan. *Arab J Chem* 2014;7:91-9.
4. Morais S, Costa FG, Pereira ML. Heavy metals and human health, Environmental Health-Emerging Issues and Practice, Prof. Jacques Oosthuizen (Ed.). ISBN: 978-953-307-854-0, In Tech. Available from: URL: <http://www.intechopen.com/books/environmental-health-emerging-issuesand-practice/heavy-metals-and-human-health>: 2012. [Last accessed on 15 Jun]
5. Barakat MA. New trends in removing heavy metals from industrial wastewater. *Arab J Chem* 2011;4:361-77.
6. Wibowo A, Rahaju FA, Iskandar, Suhartono E. The role of urinary cadmium on liver function and erythrocytes cell count in pregnancy. *Int J Biosci Biochem Bioinf* 2014;4:224-8.
7. Suhartono E, Triawanti, Yunanto A, Firdaus RT, Iskandar. Chronic cadmium hepatooxidative in rats: treatment with haruan fish (*Channa striata*) extract. *APCBEE Proc* 2013;5:441-5.
8. Asagba SO. Role of diet in absorption and toxicity of oral cadmium-a review of literature. *Afr J Biotechnol* 2009;8:7428-36.
9. Suhartono E, Triawanti, Leksono AS, Djati MS. The Role of cadmium in proteins glycation by glucose: formation of methylglyoxal and hydrogen peroxide *in vitro*. *JOMB* 2014;3:59-62.
10. Bashir N, Manoharan V, Prabu SM. Ameliorative effects of grape seed proanthocyanidins on cadmium induced metabolic alterations in rats. *Int J Biol Res* 2014;2:28-34.
11. Siddiqui AA. Exposure of cadmium chloride and toxicity stress on *Barytelphusa Gureini*. *Scholars Academic J Biosci* 2014;2:181-6.
12. Cıcık B, Engin K. The effects of cadmium on levels of glucose in serum and glycogen reserves in the liver and muscle tissues of *Cyprinus carpio* (L., 1758). *Turk J Vet Anim Sci* 2005;29:113-7.
13. Adinugroho WC, Sidiyasa K, Rostiwati T, Syamsuwida D. Ecological conditions and distribution of gemor tree species in central and east Kalimantan. *J For Res* 2011;8:50-64.
14. Arifin YF, Hamidah S, Panjaitan S, Suhartono E. *In vitro* anti-inflammatory activities of red gemor (*Nothaphoebe cf umbellifera*). *JOMB* 2015;4:312-7.
15. Santosa PB, Panjaitan S, Eryanto LJ. Gemor (*Nothaphoebe coriacea Kosterm*). HHBK in Swamp Forest; 2013.
16. Kiranmai M, Kumar CBM, Ibrahim M. Comparison of total flavanoid content of *Azadirachta indica* root bark extracts prepared by different methods of extraction. *Res J Pharm Biol Chem Sci* 2011;2:254-61.
17. Bidinotto PM, Moraes G, Souza RHS. Hepatic glycogen and glucose in eight tropical fresh water teleost fish: a procedure for field determinations of micro samples. *B Tec Cepta Pirassununga* 1997;10:53-60.
18. Suhartono E, Triawanti, Leksono AS, Djati MS. The role of cadmium in protein glycation by glucose: Formation of methylglyoxal and hydrogen peroxide *in vitro*. *JOMB* 2014;3:59-62.
19. Pandit A, Sachdeva T, Bafna P. Drug-Induced Hepatotoxicity: a review. *J Appl Pharm Sci* 2012;2:233-43.
20. Singh A, Bhat TK, Sharma OP. Clinical biochemistry of hepatotoxicity. *J Clin Toxicol* 2011;S4:1-19.
21. Duribe JO, Ogwuegbu MOC, Egwurugwu JN. Heavy metal pollution and human biotoxic effects. *Int J Phys Sci* 2007;2:112-8.
22. Viselina TN, Lukyanova ON. Change in the activity of enzymes of carbohydrate metabolism in mollusks under the influence of cadmium. *Rus J Mar Biol* 2000;26:289-91.
23. Bhati SI, Ranga D, Meena DC, Agarwal M, Chakrawarti A, Purohit RK. Ameliorative effect of *Emblca officinalis* in mice liver. *World J Pharm Res* 2014;3:846-63.
24. Al Rikabi AA, Jawad AADH. Protective effect of ethanolic gibber extract agains cadmium toxicity in male rabbits. *Bas J Vet Res* 2013;12:13-29.
25. Sobha K, Poornima A, Harini P, Veeraiah K. A study on biochemical changes in the fresh water Fish, *Catla catla* (Hamilton) exposed to the heavy metal toxicant cadmium chloride. *Kathmandu University J Sci Eng Technol* 2007;1:1-11.
26. Asagba So, Eriyamremu GE. Oral cadmium exposure alters haematological and liver function parameters of rats fed a nigerian-like diet. *J Nutr Environ Med* 2007;16:267-74.
27. Adikwu E, Deo O, Geoffrey OBP. Hepatotoxicity of cadmium and roles of mitigating agents. *Br J Pharmacol Toxicol* 2013;4:222-31.
28. Hossain MA, Mostofa MG, Burritt DJ, Fujita M. Modulation of reactive oxygen species and methylglyoxal detoxification systems by exogenous glycinebetaine and proline improves drought tolerance in mustard (*Brassica juncea L.*). *Int J Plant Biol Res* 2014;2:1-14.
29. Suhartono E, Triawanti, Leksono AS, Djati MS. Oxidative stress and kidney glycation in rats exposed cadmium. *Int J Chem Eng Appl* 2014;5:497-501.
30. Michalak A. Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. *Pol J Environ Stud* 2006;15:523-30.