



The Modulatory Effect of *Chrysobalanus icaco* seed on Cadmium Induced Hepatotoxicity and Nephrotoxicity in Rats

ABSTRACT

The fight against cadmium induced oxidative stress has been a long battle in the field of medicine, hence a lot of research is still ongoing in search of plants based on their phytochemical properties that can ameliorate cadmium induced toxicity. This study was therefore aimed at evaluating the modulatory the effect of *Chrysobalanus icaco* seed extract on some liver and kidney parameters of Wister rats exposed to cadmium. A total of twenty-four (24) rats were used for the study. The rats were divided into Four (4) groups of six (6) rats as follows: Group 1: normal control; rats in this group received distilled water daily throughout the experiment. Group 2: cadmium only; the rats in this group received 4mg/kg body weight(b wt.) of cadmium in form of Cadmium chloride, Group 3: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +100mg *Chrysobalanus icaco* (*C. icaco*) extract three times per week Group 4: the rats in this group received 4mg/kg b wt. of cadmium in form of Cadmium chloride +500mg *C. icaco* extract three times per week .The oral administration of Cadmium and *C. icaco* extract was carried out by gavage. The experiment lasted for 21 days.The results revealed that in all groups exposed to cadmium, serum level of alanine aminotransferase (ALT) 75.00 U/L, aspartate aminotransferase (AST) 145.25 U/L and alkaline phosphatase (ALP) 70.38 U/L was significantly high ($P < 0.05$) when compared to the control group (ALT (28.00 U/L),AST (75.25 U/L) and ALP (16.00 U/L)) However, serum level of ALT, AST and ALP of the Cadmium chloride + *C. icaco* extract treated groups was significantly decreased ($P < 0.05$) when compared with the non-treated cadmium group. In conclusion the study revealed that aqueous extract of *Chrysobalanus icaco* fruit is able to ameliorate cadmium-induced renal and liver dysfunction due to its anti-inflammatory and antioxidant effects.

Keywords: Cadmium,*Chrysobalanus icaco*, liver, kidney

Introduction

The presence of intrinsic elements such as cadmium in the environment is unique due to the fact that their complete removal from the environment is difficult. Cadmium is found as a contaminant in foods, cigarettes and polluted air. It is also used by industries in the production of metal coatings, plastics pigments and batteries,

(ATSDR, 2012; Satarug and Moore, 2012; Ige *et al.*, 2012; Satarug *et al.*, 2013). Hence, increase in its use in our daily lives and by industries is a major problem resulting in toxic metal pollution (Mahurpawar, 2015). In addition, the enhanced gastrointestinal and pulmonary absorption rates for cadmium makes children and women of

Corresponding Author: Kadiri, Helen Ejiro

Department of Biochemistry, Delta State University, Abraka, Nigeria.

Phone: +2348060465365; Email:hekad@yahoo.com



reproductive age more susceptible to cadmium toxicity (Meltzer *et al.*, 2010; Suh *et al.*, 2016). Indeed, increase in chronic kidney disease is a major global problem and cadmium has been found to accumulate in the kidney and very small amounts excreted from the urine (Satarug *et al.*, 2002). Studies have also indicated that no effective therapeutically metal- chelating agents, for cadmium reduction levels in kidney, has been identified. Hence kidney damage linked with cadmium is presumed to be caused from a reduction in re-absorption of nutrients by the tubules (Honda *et al.*, 2010; Wallin *et al.*, 2014). Consequently, a rise in urinary levels of these solutes is an indication of tubular malfunction. In addition, other health issues such as macular degeneration and various types of cancer has also been linked with chronic intake of cadmium in the diet (Wu *et al.*, 2014; Kim *et al.*, 2015).

Cadmium induces damage to the liver through an imbalance in cellular redox status leading to oxidative stress. However, the mechanism is not clear (Arroyo, 2012). It competes with other metals and many antioxidants proteins for their sites thereby inducing an abnormal activity as well as total loss of cellular defence by the antioxidant. (Ajayi *et al.*, 2012; Ige *et al.*, 2012; Taiwo *et al.*, 2012).

Chrysobalanus icaco L. (Chrysobalanaceae) also known as “Gbafile” or “Gbafile” by the itsekiri, is a shrub largely found in South Florida, Caribbean, South and Central America and tropical West Africa. It is traditionally used to treat fungal infections particularly in the Amazon (Ferreira-Machado *et al.*, 2014). Phytochemical studies on this plant indicates the presence of Anthocyanins, terpenes and flavonoids, such as quercetin and myricetin. (Barbosa *et al.*, 2006; Castilho, 2011; White *et al.*, 2016).

The fight against cadmium induced oxidative stress has been a long battle in the field of medicine. Several researchers have used different plants, based on the phytochemical properties they possess, in search for amelioration of cadmium

induced toxicity. Some of these plants include soybean, onion, curry and tea leaves, garlic etc (Ferramole *et al.*, 2012; Perez-Diaz *et al.*, 2013). *C. icaco* have been used in previous research to assess its ameliorative potential against several diseases and these works have been done using the leaves of this plant but there is a dearth of information on the use of its seed as an antidote to cadmium toxicity. Therefore, this research was designed to determine the effect of *Chrysobalanus icaco* on liver and kidney of rats induced with cadmium toxicity.

Materials and methods

Plant Collection and Identification

The spice *Chrysobalanus icaco* (Gbafile) was purchased from Abraka main market in Delta State, Nigeria. The Fruit was duly identified and given voucher number UBHc437 by Dr. Akinnibonsu, H.A, from the department of Botany, University of Benin, Edo State, Nigeria

Preparation of Plant Extract

The seed of *C. icaco* was removed from the pod and air dried for 10days in the laboratory. The dried seed were then oven dried and grinded to a powdered form with the aid of an electric blender. Three hundred grams (300g) of powdered leaves were soaked in 900ml of water (ratio 1:3) and the mixture was stirred every six hours for 48 hours and then filtered with sieve Whatman filter paper (size no 1) to obtain a clear filtrate which was further concentrated to dryness with hot air from the oven set at 60° C. Final extract recovered weighing 11g with percentage yield of 3.6%w/w was stored in the refrigerator before use.(El-Mahmood, 2009)

Experimental Animals

Twenty-four, 8 weeks old albino wister rats weighing between 120g and 150g were purchased from the animal house of the College of Medicine, Delta State University. The rats were fed on growers’ mash and were housed in cages constructed from aluminium steel and wire gauze under control condition of 12h light/ 12 dark

cycle. Acclimatisation was for 7 days. The Rats were handled in accordance with the laboratory practice found in the NIH (1985).

Experimental Design

The rats were divided into Four (4) groups of six (6) rats as follows:

Group 1: normal control; rats in this group received distilled water daily throughout the experiment.

Group 2: cadmium only; the rats in this group received 4mg/kg body weight (b.wt) of cadmium in form of Cadmium chloride to induce cadmium intoxication.

Group 3: the rats in this group received 4mg/kg b.wt. of cadmium in form of Cadmium chloride +100mg *C. icaco* extract three times per week.

Group 4: the rats in this group received 4mg/kg b.wt. of cadmium in form of Cadmium chloride +500mg *C. icaco* extract three times per week.

The oral administration of Cadmium and *C. icaco* extract was carried out by gavage. The experiment lasted for 21 days.

On the last day the rats were allowed to starve overnight and sacrificed by cervical decapitation. Blood was collected in to heparinised tubes, and centrifuged at a speed of 3000g for 10mins. The sera were then collected and stored frozen until needed. The liver and kidney were also extracted and 0.5g was homogenised with normal saline. The supernatant was put in empty container and stored in the fridge for biochemical analysis.

Biochemical Analysis

The L-alanine aminotransferases and L-aspartate aminotransferase activities in the serum were determined by the method of Reitman and Frankliln as modified by Gella *et al.* (1985). Alkaline Phoshatase activity was also determined in the serum by the method of Annino and Giese (1976) using the Randox kit as described in the manual. The activity of SOD in the kidney and liver was assayed for using the method of Spitz and Oberley (2001). The method of Johansson and Borg (1988) was adopted for the assay of

kidney and liver catalase activity. Lipid peroxidation was determined in the organs by using the method of Gutteridge and Wilkins (1982) Creatinine activity was determined by the method of Jung *et al.* (1987) and Urea activity was determined by the method of Zawada *et al.* (2009).

Statistical Analysis

All the experimental results were expressed as the mean \pm standard deviation. One-way analysis of variance (ANOVA) with subsequent LSD test were used to detect further difference between groups respectively, values of $p < 0.05$ were considered significant.

Table 1: Ameliorating effect of aqueous extract of *Chryobalanus icaco* on serum hepatic markers in cadmium induced Wister rats

Groups	ALT (U/L)	AST (U/L)	ALP (U/L)
Control	28.00 \pm 8.14 ^a	75.25 \pm 18.70 ^a	16.00 \pm 1.15 ^a
Cadmium control	75.00 \pm 4.00 ^b	145.25 \pm 19.36 ^b	70.38 \pm 7.53 ^b
Cadmium+100mg <i>C. icaco</i>	65.00 \pm 10.62 ^c	111.16 \pm 20.74 ^c	59.92 \pm 4.75 ^c
Cadmium + 500mg <i>C. icaco</i>	61.004 \pm 00 ^c	108.15 \pm 18.62 ^c	48.00 \pm 7.75 ^c

Values are means \pm standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p < 0.05$).

Table 2: Ameliorating effect of aqueous extract of *C. icaco* on serum renal indices in Cadmium induced Wister rats.

Groups	Urea (mg/dl)	Creatinine (mg/dl)
1-Control	15.64 \pm 3.26 ^a	1.07 \pm 0.74 ^a
2- Cadmium control	29.48 \pm 8.38 ^b	5.33 \pm 0.49 ^b
3-Cadmium+100mg <i>C. icaco</i>	23.41 \pm 2.66 ^c	2.97 \pm 0.09 ^c
4-Cadmium+500mg <i>C. icaco</i>	18.41 \pm 3.66 ^a	1.97 \pm 0.09 ^a

Values are means \pm standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly ($p < 0.05$).

Table 3: Ameliorating effect of aqueous extract of *Chryobalanusicaco* on hepatic antioxidant markers in cadmium induced Wister rats

Groups	SOD (Units/gtissue)	CAT (Units/gtissue)	MDA (Units/gtissue)
1-Control	42.25 ± 1.72 ^a	34.18 ± 4.42 ^a	0.29 ± 0.19 ^a
2- Cadmium control	26.34 ± 0.54 ^b	17.23 ± 1.60 ^b	0.81 ± 0.35 ^b
3-Cadmium+100 mg <i>C.icaco</i>	35.64 ± 4.08 ^c	22.65 ± 1.90 ^c	0.41 ± 0.14 ^c
4-Cadmium+500 mg <i>C.icaco</i>	38.34± 4.08 ^c	27.65 ± 1.10 ^c	0.36 ± 0.19 ^c

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).

Table 4: Ameliorating effect of aqueous extract of *Chryobalanusicaco* on renal antioxidant markers in cadmium intoxicated Wister rats.

Groups	SOD (Units/gtissue)	CAT (Units/gtissue)	MDA (Units/gtissue)
1-Control	38.18 ± 7.01 ^a	25.21 ± 3.75 ^a	0.13 ± 0.02 ^a
2- Cadmium control	26.94 ± 5.81 ^b	11.78 ± 4.76 ^b	0.63 ± 0.08 ^b
3-Cadmium+100mgCi	32.38 ± 2.15 ^a	19.15 ± 0.82 ^c	0.31 ± 0.08 ^c
4-Cadmium+500mgCi	34.27± 5.51 ^{a,c}	21.45 ± 2.75 ^c	0.27 ± 0.02 ^c

Values are means ± standard deviations of triplicate determinations. Values not sharing a common superscript on the same column differ significantly (p<0.05).

Discussion

Cadmium has been recognized as one of the most toxic environmental and industrial pollutants. Its induced renal toxicity was reported earlier by Patra *et al.* (2011); Imafidon *et al.* (2015). Mallya *et al.* (2017).

L-alanine aminotransferases (ALT) and L-aspartate aminotransferase (AST) are well known indicators of liver damage. (Al-Habori *et al.*, 2002). The result of this study (Table 1) showed that liver dysfunctions was induced by oral exposure of rats to sub lethal concentration of cadmium as demonstrated by a significant increase in serum

ALT, AST and ALP. This is consistence with the reports of Bharav *et al.* (2010); Onoshe (2014) and Imafidon *et al.* (2015). This damage may be due to direct toxic effects of the cadmium on the hepatocytes, since the liver is the site of detoxification of all types of toxins and chemicals (Adefegha *et al.*, 2015).

Recent researches have been on counteracting heavy-metal-induced toxicity by use of plant-derived components or extracts with antioxidant potential (Xu *et al.*, 2017). This present study showed that the administration of the aqueous extract of *C. icaco* reduced cadmium induced liver

dysfunction as verified by lowered, ALT, ALP and AST levels (Table 1), in the cadmium treated rats.

One major function of the kidney is clearance of metabolites from the blood (Miners, 2017) and agents with nephrotoxicity effects may lead to a raise in serum urea level. The result of this present study indicates a significant increase in creatinine and urea levels in the serum of the rats exposed to cadmium only (group 2) as compared with the normal control (Table 2). This elevated serum urea and creatinine may be due to an imbalance in the kidney resulting in an increase in urea and creatinine levels as well as oxidative damage to the kidney (Tarasub *et al.*, 2011; Adefegha *et al.*, 2015). This is in line with previous works conducted by Poontawee *et al.* (2016) who investigated the protective effect of *Cleistocalyx nervosum* var. *paniala* fruit extract against oxidative renal damage caused by cadmium and Jafarpour *et al.* (2017). However, treatment of the rats with different doses of the extracts (Groups 3 and 4) as seen in Table 2 significantly reduced Serum Creatinine and urea levels when compared with the untreated Group 2 rats. Indicating that *C. icaco* as a nephroprotective effect.

Lipid peroxidation levels (MDA) was also significantly increased in the liver and kidney of the Cd treatment groups compared to the control groups (Table 3 and 4). This is in line with previous research by Elmallah *et al.* (2017) where they reported an increase in lipid peroxidation in the testes of rats treated with cadmium. The increased Lipid peroxidation in this present study (Table 3 and 4) may be due to decreased activities of Superoxide

dismutase (SOD) and Catalase (CAT), the free radical scavenging enzymes as stated by Atagana and Asagba (2015) in their study on the protective effects of symbiotic diets of *Bacillus coagulans*, *Lactobacillus plantarum* and *inulin* against acute cadmium toxicity in rat.

A significant decrease ($p < 0.05$) was also indicated in the CAT and SOD activity of the liver and kidney of rats intoxicated with cadmium when compared with the control (Table 3 and 4) indicating a damage to these organs. These finding are consistent with that of Atagana and Asagba (2015) and Elmallah *et al.* (2017) who recorded similar results in their work on cadmium induced toxicity in rats. Patra *et al.* (2011) also stated in earlier studies that significant decrease in catalase and super oxide dismutase could be due to the fact that they are part of the three endogenous antioxidant enzymes responsible for the removal of reactive oxygen species and needed in the protection of cells and tissues from oxidative damage.

However, treatment of the cadmium intoxicated rats with the aqueous extract of *C. icaco* reversed these trends. This confirms the antioxidant property of this spice due to its rich antioxidants content (Castilho 2011; White *et al.* 2016). Previous phytochemical studies of *C. icaco* demonstrated that it contains phytochemicals (Fernandes *et al.*, 2003; Castilho and Kaplan, 2011). Chemical analysis of *C. icaco* extract identified delphinidin, anthocyanins, cyanidin, petunidin and peonidin derivatives as major components (Venancio *et al.*, 2016). Several studies have also reported that flavonoids have anti-inflammatory and antioxidant effects (Onoche,

2014) and these antioxidants are the frontline of defence against free radicals (Onoche, 2014). It can therefore be suggested that the role of the aqueous extract of *Chrysobalanus icaco* fruit in ameliorating cadmium-induced renal and liver dysfunction as observed in this study is due to the anti-inflammatory and antioxidant effects of the different compounds of the seed.

Conclusion

The result in this study confirms that *Chrysobalanus icaco* seed has antioxidant properties and is able to protect the kidney from damage caused by cadmium toxicity in rats, which can also be inferred in man.

REFERENCES

- Adefegha, S. A., Omojokun, O. S. and Oboh, G. (2015). Modulatory effect of protocatechuic acid on cadmium induced nephrotoxicity and hepatotoxicity in rats in vivo. *SpringerPlus*, 4, 619.
- Agency for Toxic Substances and Disease Registry (ATSDR) (2012). Toxicological profile for cadmium. Department of Health and Human Services, Public Health Service, Centers for Disease Control, Atlanta, GA, USA.
- Ajayi, A.F., Akhigbe, R.E., Adewumi, O.M., and Olaleye, S.B. (2012). Haematological evaluation of *Cryptolepis sanguinolenta* stem ethanolic extract in rats. *International Journal of Medicine and Biomedical Research*, 1: 56–61
- Al-Habori, M., Al-Aghbari, A., Al-Mamary, M. and Baker, M. (2002) Toxicological evaluation of *Cathaedulis* leaves. A longterm feeding experiment in animals. *Journal of Ethnopharmacology*, 83:209– 217.
- Annino, J.S. and Giese, R.W. (1976). Clinical chemistry, Principles and Procedures (4thedn) Little brown and company Boston, pp 252-265.
- Arroyo, V.S., Flores, K.M., Ortiz, L.B, Gómez-Quiroz, L.E. and Gutiérrez-Ruiz, M.C. (2012). Liver and Cadmium Toxicity. *Journal of Drug and Metabolic Toxicology*, 5:001.
- Atagana, O.S. and Asagba, S.O. (2015). Protective effects of honey against cadmium-induced alteration of some biochemical parameters in rats. *Journal of Toxicology and Environmental Chemistry*, 96(10):1557-1563.
- Barbosa, W.L., Peres, A., Gallori, S. and Vincieri, F.F. (2006). Determination of myricetin derivatives in *Chrysobalanus icaco* L. (*Chrysobalanaceae*) Brazilian *Journal of Pharmacognosy*, 16:333–7.
- Bharavi, K., GopalaReddy, A., Rao, G.S., Rajasekhara Reddy, A. and Rama Rao, S.V. (2010). Reversal of Cadmium-induced Oxidative Stress in Chicken by Herbal Adaptogens *Withania Somnifera* and *Ocimum Sanctum*. *Toxicology International*, 17(2): 59–63.
- Castilho, R.O., Souza, I., de Guimarães, U.P. and Kaplan, M.A.C. (2000). A survey of chemistry and biological activities of *Chrysobalanaceae*. *Anna Academic Bras. Cienc.* 72 Sessions of the Academia Brasileira de Ciências, 293.
- Elmallah, M., Elkhadragey, M. F., Al-Olayan, E. M., and Abdel Moneim, A. E. (2017). Protective Effect of *Fragaria ananassa* Crude Extract on Cadmium-Induced Lipid Peroxidation, Antioxidant Enzymes Suppression, and Apoptosis in Rat Testes. *International Journal of molecular sciences*, 18(5), 957.
- El-Mahmood, M (2009). Antibacterial activity of crude extracts of *Euphorbia hirta* against some bacterial associated enteric infections. *Journal of Ethnopharmacology*, 95:277-284.
- Ferramola, M.L., Pérez-Díaz, M.F., Honoré, S.M., Sánchez, S.S., Antón, R.I., Anzulovich, A.C and Giménez, M.S. (2012). Cadmium-induced oxidative stress and histological damage in the myocardium: Effects of a soy-based diet *Toxicology and Applied Pharmacology*, 265: 380–389.
- Ferreira-Machado, SC., R.F., Gagliardi, R.F., Nunes, A.P.M., Rodrigues, M. P., Dantas, F.J.S. De, Mattos, J. C. P., Peregrino, C.A.F. Moura, E.G. and Caldeira-de-Araujo, A. (2014). Antidiabetic and genotoxic effects on Wistar rats treated with aqueous extract from *Chrysobalanus icaco* L. *Journal of Medicinal plants*, 81(1):52-57.
- Gella F.J., Olivella T., Pastor M.C., Arenas J., Moreno R., Durban R. and Gomez J.A. (1985). A simple procedure for the routine determination of aspartate aminotransferase and alanine aminotransferase with

- pyridoxal phosphate. *Clinical and Chemical Acta*, 153:241–247.
- Gutteridge, J.M.C. and Wilkins, C. (1982). Copper dependent hydroxyl radical damage to ascorbic acid. Formation of thiobarbituric acid reactive product *FEBS Letters.*, 137: 327-340.
- Honda, R., Swaddiwudhipong, W., Nishijo, M., Mahasakpan, P., Teeyakasem, W., Ruangyuttikarn, W., Satarug, S., Padungtod, C. and Nakagawa, H. (2010). Cadmium induced renal dysfunction among residents of rice farming area downstream from a zinc-mineralized belt in Thailand. *Toxicological Letters*, 198: 26-32.
- Ige, S.F., Akhigbe, R.E., Edeogho, O., Ajao, F.O., Owolabi, O.Q. and Oyekunle, OS. (2011). Hepatoprotective activities of *allium cepa* in cadmium-treated rats. *International Journal of Pharmacy and Pharmaceutical Science*, 3(5):60–3.
- Kaplan, J. H. and Groves, J. N. (1972) *Cancer Research.*, 32, 1190.
- Imafidon, C.E.R., Akomolafe, R.O., Abubakar, S.A., Olukiran, O.S. and Ayowole O.A. (2015). Amelioration of Cadmium-Induced Nephropathy using Polyphenol-rich Extract of *Vernonia amygdalina* (Del.) Leaves in Rat Model. *Open Access Macedonia Journal of Medical Science*, 13(4): 567–577.
- Jafarpour, D., Shekarforoush, S. S., Ghaisari, H. R., Nazifi, S., Sajedianfard, J. and Eskandari, M. H. (2017). Protective effects of synbiotic diets of *Bacillus coagulans*, *Lactobacillus plantarum* and inulin against acute cadmium toxicity in rats. *BMC complementary and alternative medicine*, 17(1), 291. doi:10.1186/s12906-017-1803-3.
- Johansson, L.H. and Borg, L.A. (1988). A spectrophotometric method for determination of catalase activity in small tissue sample. *Annals of Biochemistry* 174(1):331-336
- Jung, K., Wesslau, C., Priem, F., Schreiber, G and Zubek, A. (1987). Specific creatinine determination in laboratory animals using the new enzymatic test kit, “Creatinine-PAP,” *Journal of Clinical Chemistry and Clinical Biochemistry*, 25(6): 357–361.
- Kim, N.H., Hyun, Y.Y., Lee, K.B., Chang, Y., Ryu, S., Oh, K.H. and Ahn, C. (2015) Environmental heavy metal exposure and chronic kidney disease in the general population. *Journal of Korean Medical Science*, 30: 272-277.
- Mahurpawar, M. (2015). Effects of heavy metals on human health. Social issues and environmental problems. *International Journal of Research*, 3 (9):7234
- Mallya, R., Chatterjee, P., NAVinodini, K. Poulomi, C. and Prasanna, M. (2017). *Moringa oleifera* leaf extract: beneficial effects on cadmium induced toxicities - a review. *Journal of Clinical and Diagnostic Research*, 11(4): CE01-CE04
- Miners, J.O., Yang, X., Knights, K.M. and Zhang, L. (2017) The Role of the Kidney in Drug Elimination: Transport, Metabolism, and the Impact of Kidney Disease on Drug Clearance *Clinical pharmacology and Therapeutics*. 102(3):436-449.
- Meltzer, H.M., Brantsaeter, A.L., Borch-Johnsen, B., Ellingsen, D.G., Alexander, J., Thomassen, Y., Stigum, H. and Ydersbond, T.A. (2010). Low iron stores are related to higher blood concentrations of manganese, cobalt and cadmium in non-smoking, Norwegian women in the HUNT 2 study. *Environmental Research*, 110: 497-504.
- National Institutes of Health; NIH (1985). Guide for the care and use of laboratory animals NIH Publication No. 85-23. Revised
- Onoshe, S. and Madusolumuo M.A. (2014). Effect of hexane seed extract of *Nigella sativa* on cadmium induced renal dysfunction in rats. *American Journal of Research Communication*, 2(1): 158-171.
- Patra, R. C., Rautray, A. K. and Swarup, D. (2011). Oxidative Stress in Lead and Cadmium Toxicity and Its Amelioration. *Veterinary Medicine International*, 1:9.
- Pérez Díaz, M.F., Acosta, M., Mohamed, F.H., Ferramola, M.L., Oliveros, L.B., and Gimenez, M.S. (2013). Protective effect of soybeans as protein source in the diet against cadmium-aorta redox and morphological alteration. *Toxicology and Applied Pharmacology*, 272: 806–815.
- Poontawee, W., Natakankitkul, S. and Wongmekiat, O. (2016). Protective Effect of *Cleistocalyx nervosum* var. paniala Fruit Extract against Oxidative Renal Damage Caused by Cadmium. *Molecules*, 21(2), 133.
- Satarug, S., Baker, J.R., Reilly, P.E., Moore, M.R. and Williams, D.J. (2002) Cadmium levels in the lung, liver, kidney cortex, and urine samples from Australians without occupational exposure to metals. *Archives of Environmental Health*, 57: 69-77.

- Satarug, S. and Moore, M.R. (2012). Emerging roles of cadmium and heme oxygenase in type-2 diabetes and cancer susceptibility. *Tohoku Journal of Experimental Medicine* 228, 267-288.
- Satarug, S., Swaddiwudhipong, W., Ruangyuttikarn, W., Nishijo, M. and Ruiz, P. (2013) Modeling cadmium exposures in low- and high-exposure areas in Thailand. *Environmental Health Perspective* 121: 531-536
- Spitz, D.R. and Oberley, L.W. (2001). Measurement of MnSOD and CuZnSOD activity in mammalian tissue homogenates. *Current Protocols in Toxicology*. 8:1-11
- Suh, Y. J., Lee, J.E., Lee, D.H., Yi, H.G., Lee, M.H., Kim, C.S., Nah, J.W. and Kim, S.K. (2016). Prevalence and relationships of iron deficiency anaemia with blood cadmium and vitamin D levels in Korean women. *Journal of Korean Medical Science*, 31:25-32.
- Taiwo, I.A., Adewumi, O.O. and Odeigah, P.G.C. (2012). Assessment of *Bridelia ferruginea* benth for its therapeutic potential in pregnancy-induced impaired glucose tolerance in rats. *International Journal of Medicine and Biomedical Research*, 1: 49-55
- Tarasub, N., Tarasub, C. and Na Ayutthaya, W.D. (2011). Protective role of curcumin on cadmium-induced nephrotoxicity in rats. *Journal of Environmental Chemistry and Ecotoxicology*, 3(2):17-24.
- Wallin, M., Sallsten, G., Lundh, T. and Barregard, L. (2014). Low-level cadmium exposure and effects on kidney function. *Occupation and Environmental Medicine*, 71: 848-854.
- White P.A., Cercato, L.M., Batista, V.S., Camargo, E.A., De Lucca, W. and Jr, Oliveira, A.S. (2016). Aqueous extract of *Chrysobalanus icaco* leaves, in lower doses, prevent fat gain in obese high-fat fed mice. *Journal of Ethnopharmacology*, 179:92-100.
- Venancio V.P., Marques, M.C., Almeida, M.R., Mariutti, L.R. and Souza, V.C. (2016) *Chrysobalanus icaco* L. fruits inhibit NADPH oxidase complex and protect DNA against doxorubicin-induced damage in Wistar male rats. *Journal of Toxicology and Environmental Health A* 79(20):885-893.
- Wu, C.C., Gupta, T., Garcia, V., Ding, Y. and Schwartzman, M.L. (2014a). 20-HETE and blood pressure regulation: clinical implications. *Cardiology Review*, 22: 1-12.
- Xu, D. P., Li, Y., Meng, X., Zhou, T., Zhou, Y., Zheng, J.J and Li, H. B. (2017). Natural Antioxidants in Foods and Medicinal Plants: Extraction, Assessment and Resources. *International journal of molecular sciences*, 18(1), 96.
- Zawada, R. J., Kwan, P., Olszewski, K. L., Llinas, M., and Huang, S. G. (2009). Quantitative determination of urea concentrations in cell culture medium. *Biochemistry and cell biology*, 87(3), 541-544.