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Research Article

Hepatoprotective effects of palm oil and coconut water in the serum of Wistar rats exposed to cadmium chloride contaminated diet.

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ABSTRACT: To study the hepatoprotective effects of palm oil and coconut liquor in the liver, twenty four Wistar rats were exposed to moderate level of cadmium chloride for 6 weeks. The rats were divided into four Groups of 6 rats each. Group I animals received distilled water and a diet free of $CdCl_2$ while Group II animals were given $CdCl_2$ contaminated diet of 50mg/kg diet. Also, Group III and IV animals were administered 3ml palm oil and 3ml coconut water respectively along side fed with a $CdCl_2$ contaminated diet of 50mg/kg diet for the same duration. The palm oil and coconut water were given orally once daily by gavage. $CdCl_2$ caused an elevated increase in the levels of ALT, AST and ALP which was significantly ($p<0.05$) different from the control and other Groups. On the contrary, palm oil and coconut water decreased ALT, AST and ALP to a significantly ($p<0.05$) level. There was also a drop in the protein level of the $CdCl_2$ administered Group alone when compared with the palm oil and coconut water Groups, indicating loss of hepatocytes. In conclusion, this study reveals that palm oil and coconut liquor possess properties that can be able to ameliorate $CdCl_2$- induced toxicity (which is dose dependent) and this supports its usage in local treatment as an antidote upon the accidental consumption of some xenobiotics.

KEYWORDS: Cadmium chloride ($CdCl_2$), coconut water, hepatoprotective, palm oil.

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INTRODUCTION

Food contamination has become a big problem in the world today (WHO, 1992). It refers to the presence of harmful chemicals and microorganisms which can cause consumer illness. The impact of chemical contaminants on consumer health and well-being is often apparent only after many years of processing prolonged exposure at low levels (Okorie *et al*., 2014). Chemical contaminants present in foods are often unaffected by thermal processing unlike most microbiological agents (Valdes and Ziobro, 2000).

One common chemical contaminant of food is cadmium chloride. Cadmium chloride is a metal salts that is widely used in industries. It is a toxicologically important contaminant which accumulates in the body and inhibits a number of enzyme containing sulfhydral Groups (Rhman *et al*. 2011; Latinwo, 1997). It is made up of cadmium and chloride with the formula, $CdCl_2$. Cadmium is a chemical element with the symbol Cd and atomic number 48. This soft, bluish-white metal is chemically similar to the two other stable metals in Group 12, zinc and mercury. Like zinc, it prefers oxidation state +2 in most of its compounds and like mercury it shows a low melting point compared to transition metals. Cadmium is also an environmental hazard. There have been a few instances of general population toxicity as the result of long-term exposure to cadmium in contaminated food and water, and research is ongoing regarding the estrogen mimicry that may induce breast cancer (Rhman *et al*., 2011; Mann, 2012). It was reported that the maximum tolerable dietary $CdCl_2$ level for domestic animals was 0.5ppm. Dietary concentrations of 1ppm result in undesirable effects while 5ppm caused adverse health effects (Manca *et al.,* 1991; McDowell, 1992).

Most natural substances like herbs and fruits have been and are still being tested in experiments to discover their effects in xenobiotic management in the biological system (Sirajudeen *et al*., 2006). Some examples of these substances are coconut water and palm oil.

Coconut water, the clear liquid endosperm obtained from immature coconuts, is a refreshing and nutritious beverage, widely consumed around the world due to its beneficial health properties. (Pummer *et al.,*2001; Paniappan, 2002).In addition to protect against induction of myocardial infarction (Anurag and Rajamohan, 2003), coconuts water plays an important role for oral rehydration and even for intravenous hydration of patients in remote regions (Campkell-Falck *et al*, 2000; Barclay, 2011).Results obtained from one study suggest that tender coconut water (TCW) treatment could prevent and reverse high blood pressure induced by high fructose diet probably by inhibition of lipid peroxidation, upregulation of antioxidant status and improved insulin sensitivity (Bhagya *et al.,* 2010).

Palm oil (also known as dendê oil, from Portuguese) is an edible vegetable oil derived from the mesocarp (reddish pulp) of the fruit of the oil palms, primarily the African oil palm *Elaeis guineensis* (Reeves *et al*., 1979), and to a lesser extent from the American oil palm *Elaeis oleiifera* and the Maripa palm *Attalea maripa*. The benefit of palm oil in both the industry and in medicine cannot be overemphasized. The presence of high amount of Vitamin E (α tocopherol) in palm oil has shown positive health benefits. Tocotrienols from red oil is a potent inhibitor of lipid peroxidation and protein oxidation (Kamat and Devasagayam, 1995).

Locally in some part of Delta State, Nigeria (*Uhrobo* land); palm oil and coconut water are used as first line treatment when poisonous, toxic substances or xenobiotics (such as kerosene or crude oil products) are accidentally consumed by individuals. It is on the bases of this folk assumption that this present study attempts to evaluate the hepatoprotective effect of palm oil and coconut liquor on some serum biomarkers of liver damage in rats fed on a moderate dose of cadmium chloride contaminated diet.

MATERIALS AND METHODS

Collection Plant Material

Palm Oil: In other to avoid adulteration, fresh palm oil used was purchased from the Okitipupa Oil Palm Mill Ltd, Ondo State, Nigeria.

Coconut liquor: Coconut water from mature coconuts (*Cocosnucifera* L., Arecaceae) of 10-12 months of age, (West Coast Tall variety) grown in the University campus were used for this study. The coconuts were dehusked and liquid endosperm was collected filtered and pooled. The pooled mature coconut water was stored at 0- 4 ºC and used for the experiment. It was freshly reconstituted with distilled water prior to administration to rats.

Experimental Animal

Twenty-four white albino rats (Wistar strain, weighing 160-200 g) used for this study was purchased from the Animal Unit, College of Medicine, Ambrose Ali University, Ekpoma, Edo state, Nigeria .They were divided into four experimental Groups of six rats per Group. Member of each Group were housed in standard rat cages (Griffin and George Modular Cage System; model YSM 580 cage base and YSM 600-540 cage top) and allowed to acclimatize to laboratory conditions for 7days before the commencement of the experiment. All rats were allowed access to drinking water and chow (feed)-product of Edo Feeds and Flour Mill (EFFM), EWU, Edo State, Nigeria. Permission and approval for animal studies were obtained from the College of Health Sciences Animal ethics committee, Delta State University, Abraka. This study was carried out between the periods of October – December, 2014.

Animal Treatment

Animals in Group I which served as the positive control were fed normal diet and distilled water only ($CdCl_2$ free) for 6 weeks. Rats in Group II received cadmium contaminated diet

(50 mg $CdCl_2$/Kg diet) alone for 6 weeks. Group III animals were given orally by gavage, 3ml Palm oil/ Kg body weight once daily for same period (at the early hours of the day),before the commencement of feeding with cadmium contaminated diet (50 mg $CdCl_2$/Kg diet). For the same duration and in the same manner, rats in IV received 3ml Coconut water/ Kg body weight and 50 mg $CdCl_2$/Kg diet. All animals were allowed access to drinking water.

Average body weight and body weight gain was measured weekly for each Group. Also, the amount of fed left over, were collected and measured. The administration of moderate dose of 50 mg $CdCl_2$/Kg diet was adopted as described by Noël *et al*., 2004.

Preparation of Serum and tissues homogenate:

Each rat was anaesthetized in di-ethyl ether saturated chamber and under such condition; the thoracic and abdominal regions were opened to expose the liver and kidney. Blood was obtained via heart puncture by means of a 5ml disposable hypodermic syringe and needle and placed in plain to clot in ice-cold bottles. The blood samples were centrifuged at 1000x g (Uniscope Model SM 902B Bench Centrifuge) for 10 minutes at room temperature in order to obtain serum samples. They were collected and stored frozen until required for assay which was performed within 48 hours.

Biochemical Assay

The biochemical investigation of the samples was carried out with the following commercially available kits as supplied by TECO Diagnostic, Anaheim, USA. Alkaline phosphatase (ALP) as described by Tietz and Shuey (1986), aspartate aminotransferase (AST) (Bergmeyer *et al*., 1985) and alanine aminotransferase (ALT) (Klauke *et al*., 1993) were adopted. Also, serum protein determination was examined by following the method of Lowry et al., (1951) using bovine serum albumin (BSA), at 660nm.

Statistical analysis

One-way ANOVA with Dunnett's post test was performed using GrapPad Prism version 4.00 for Windows, GraphPad software, San Diego California USA. Difference were considered to be statistically significant at $p<0.05$.

RESULTS

After 6 weeks of administration, there was no death or physical sign of toxicity in all the animals throughout the period of study, except for the reduced activity in some animals in Group II ($CdCl_2$ alone). A significant ($p<0.05$) reduction in food intake was observed for the cadmium chloride treated Group alone when compared with the control and other Groups (Table 1). Although a significant reduction in food intake was observed for the palm oil and coconut treated Groups respectively when compared with the control, this was significantly higher than that observed for the Cd administered Group alone (Table 1). Result from table 1 also revealed a significant ($p<0.05$) decrease in body weight again for all cadmium treated Groups when compared to the control.

The result from Table 2 showed that the administration of cadmium chloride caused a significant ($p<0.05$) increase in serum alanine aminotransferase (ALT), aspartate aminotransferase (AST) and alkaline phosphatase (ALP) when compared with the control rats. However, animals pre-treated with palm oil and coconut liquor significantly reduced these enzymes below levels that can cause hepatic damage. Similarly, serum albumin in $CdCl_2$treated Group alone was significantly ($p<0.05$) reduced compared with the control and animals that were pre-treated with palm oil and coconut water (Table 2).

DISCUSSION

Cadmium toxicity is directly related to cadmium absorption and retention in some tissues such as liver, kidney and gastrointestinal tracts (Rhman *et al*, 2011).Following oral administration, the most common effects induced by short-term exposure to cadmium chloride are reduced growth, alterations in organ weights or histopathology (particularly of the kidney, testes, liver, and intestine), and effects on the immune system (Borzelleca *et al*., 1989).

The liver is a major site for xenobiotic metabolism and is thus prone to oxidative damage by toxicants, pro-drugs or pro-carcinogens. The highest concentrations of cadmium in the human body accumulate in the kidney (particularly the cortex) and the liver, although the metal can be detected in virtually all tissues (Elinder, 1985). The result from this study (Table 1) indicated that, after 6 weeks of administration, a reduced feed intake as well as reduced weight gain in the cadmium chloride treated Group alone was observed when compared with the control and other Groups. Although palm oil and coconut liquor showed a significant ($p<0.05$) increase in feed intake per week and body weight gain when compared with the $CdCl_2$ Group, the value obtained was significantly ($p<0.05$) lower from the control Group. Previous studies has shown that high dietary levels of Cd results in suppressed feed intake and weight gain, reduction in bone mineralization and anaemia (Vallec and Ulmer, 1972; WHO, 1992).The reason for this decrease in weight gain and feed intake by $CdCl_2$may not be clear but previous studies have suggested that $CdCl_2$ might interfere with the absorption and metabolism of some dietary nutrient because of its solubility (Vallec and Ulmer, 1972). Also, the biochemical alteration which occurs prior to morphological changes in the organs and the changes in certain enzyme levels in extracellular fluids may reflect the extent of Cd-induced damage in target organs (Khandelwal *et al*., 1991, Saskia *et al*., 2014). Palm oil and coconut liquor showed positive effects in ameliorating the toxicity of Cd by improving weight loss (Table 1).

Table 1: Mean intake and change in body weight and body weight gain in rats fed with cadmium chloride contaminated diet.

Treatment (g/kg)	Food intake (g/weeks/rat)	Body Weight (g)	Body Weight gain (g)
Group I (Control)	212.3 ± 4.11	199 ± 5.1	50 ± 1.6
Group II ($CdCl_2$/kg diet) only	159.4 ± 1.11**	147 ± 3.3**	17 ± 3.5**
Group III ($CdCl_2$/kg + 3ml Palm oil/kg)	188. 5 ± 0.94*	166 ± 1.8*	29 ± 3.1*
Group IV ($CdCl_2$/kg + 3ml Coconut water/kg)	179.7 ± 1.20*	162 ± 2.5*	31 ± 2.9*

Values are expressed as Mean ± SD of 6 rats per group (n=6). p< 0.05 values are considered to be statistically significant. Values with the superscript ** are significantly different from the control and other groups. Values with * are significantly different from control.

Table 2: The effect of Palm oil and Coconut liquor on Cadmium Chloride contaminated diet.

Treatment (g/kg)	ALT (IUL^{-1})	AST (IUL^{-1})	ALP (IUL^{-1})	Albumin (mg/ml)
Group I (Control)	52.5 ± 3.5	15.7 ± 0.9	133.8 ± 5.5	4.02 ± 1.2
Group II ($CdCl_2$/Kg diet) only	111.3 ± 2.3**	30.2 ± 1.2**	222 .3 ± 11.7**	2.03± 3.1**
Group III ($CdCl_2$/Kg + 3ml Palm oil/kg)	89.0 ± 2.7*	23.5 ± 2.3*	135.1 ± 6.0	3.86 ± 2.9
Group IV ($CdCl_2$/Kg + 3ml Coconut water/kg)	88.8 ± 1.9*	21.1 ± 1.9*	137.4 ± 5.7	4.49 ± 1.6

Values are expressed as Mean ± SD of 6 rats per group (n=6). p< 0.05 values are considered to be statistically significant. Values with the superscript ** are significantly different from the control and other groups. Values with * are significantly different from control.

In the assessment of liver damage by cadmium chloride, the determination of enzyme marker levels such as ALT and AST is often used. In necrosis or membrane damage, the enzymes are released into circulation and it can therefore be measured in serum as markers of hepatic damage. High levels of AST and ALT indicates liver damage, such as in viral hepatitis as well as cardiac infarction and muscles injury (Moss and Butterworth, 1974). Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in liver (Drotman and Lawhorn, 1978, Saskia *et al*., 2014). Similarly, serum ALP level on the other hand is related to the function of hepatic cell.

In this study, we demonstrated the effectiveness of palm oil and coconut liquor by the administration of $CdCl_2$ contaminated diet in rats, which is a known model for hepatic injury. Although the liver has a good number of defence mechanisms against oxidative stress caused by toxicants, long term exposure to these toxicants as well as increased concentration might deplete the level of protection hence leading to liver damage (Oyabemi and Odetola, 2010).

Results from Table 2 revealed significant increases in the levels of AST, ALT, and ALP in the $CdCl_2$ treated Group alone when compared with the control and other Groups. The elevated serum liver enzymes can be attributed to the damage in the histostructural integrity of the liver hepatocytes (Kaplowitz, 2001). Furthermore, the palm oil and coconut water Groups showed significantly reduced level in AST, ALT and ALP when compared with the $CdCl_2$ Group alone. The abnormal high level of serum ALT, AST and ALP observed in this study (Table 2) are the consequences of $CdCl_2$ induced liver damage to the hepatocytes. Treatment with palm oil and coconut water respectively reduced the enhanced level of serum ALT, AST and ALP, which seems to offer protection and maintain the functional integrity of hepatic cells.

Previous research has shown that palm oil and coconut water prevents lipid peroxidation of cells especially in the liver because they possess antioxidant (Prathapan and Rajamohan, 2011; Preetha *et al.*, 2012; Preetha *et al*., 2013; Kamat and Devasagayam, 1995). Also, glutathione peroxidase activity has being shown to significantly increase in the liver of rats with fed palm oil supplemented diet (Oluba

et al., 2008). Beyond the unique lipid profile of palm oil, there is growing scientific interest in the lipophilic palm oil–associated tocol members of the vitamin E family that possess potent nutritional and therapeutic value (Kamat and Devasagayam, 1995; Sen *et al*., 2007, Sen *et al*., 2010). Palm oil derived from *Elaeis guineensis* represents the richest source of the lesser characterized vitamin E, α-tocotrienol. One of 8 naturally occurring and chemically distinct vitamin E analogs, α-tocotrienol possesses unique biological activity that is independent of its potent antioxidant capacity (Sen *et al*., 2007, Sen *et al*., 2010). Current developments in α-tocotrienol research demonstrate neuroprotective properties for the lipid-soluble vitamin in brain tissue rich in polyunsaturated fatty acids (PUFAs). Arachidonic acid (AA), one of the most abundant PUFAs of the central nervous system, is highly susceptible to oxidative metabolism under pathologic conditions. Cleaved from the membrane phospholipid bilayer by cytosolic phospholipase A_2, AA is metabolized by both enzymatic and non-enzymatic pathways. A number of neurodegenerative conditions in the human brain are associated with disturbed PUFA metabolism of AA, including acute ischemic stroke (Sen *et al*., 2010). Palm oil–derived α-tocotrienol at nanomolar concentrations has been shown to attenuate both enzymatic and nonenzymatic mediators of AA metabolism and neuro-degeneration (Sen *et al*., 2010).

Studies have revealed that coconut water treatment could prevent and reverse high blood pressure induced by high fructose diet probably by inhibition of lipid peroxidation, upregulation of antioxidant status and improved insulin sensitivity (Bhagya, *et al.,* 2010; Preetha *et al.*, 2013).

The chemical composition and biological properties of coconut (*Cocos nucifera* L.) water, was shown to contain phytohormone; auxin; cytokinin; gibberellin; inorganic ion; and vitamin (Yong *et al*, 2009). Phytohormones are a Group of naturally occurring organic compounds that play crucial roles in regulating plant growth in a wide range of developmental processes. Initially, the term phytohormone was synonymous with auxin. Later on, the other plant growth regulators such as gibberellins (GAs), ethylene, cytokinins, and abscisic acid (ABA) were categorized together with auxins as the “classical five” hormones (Kende and Zeevaart, 1997). Coconut water contains auxin, various cytokinins, GAs and ABA (Yong *et al*, 2009; Kobayashi *et al*., 1997; Wu and Hu, 2009; Ma *et al*., 2008).

Vitamins and inorganic ions (such as calcium, sodium potassium and magnesium) which are essential for the normal functioning of the human body are also found in coconut water (IOM, 2000). Greater consumption of fruits and vegetables is associated with the reduced risk of cardiovascular disease, stroke, and cancers of the mouth, pharynx, esophagus, lungs, stomach, and colon (Riboli and Norat, 2003; Bazzano, 2002) because they contain vitamins and minerals vital for normal physiological functions (Tucker and Roberts, 2000). Coconut water contains vitamins B1, B2, B3, B5, B6, B7 and B9 (Yong *et al*, 2009). The B vitamins are water-soluble and are required as coenzymes for enzymatic reactions essential for cellular function (Depeint, 2006). In addition to vitamin B, coconut water also contains vitamin C (Yong *et al*, 2009), which is an important dietary antioxidant (Shenkin, 2006). The presence of these ions and vitamins might have contributed to the therapeutic value inherent in coconut water.

A reduction in serum protein (albumin) observed in the $CdCl_2$ treated rats (Table 2), may be associated with the decrease in the number of hepatocytes which in turn may result into decreased hepatic capacity to synthesize protein and consequently decrease in the liver weight. However, the administration of palm oil and coconut water revealed an increase in the level of albumin (Table 2), an indication of reduced hepatocyte degeneration or probably hepatic cell regeneration.

Conclusion

The outcome of this study however demonstrates that palm oil and coconut water has some protective effect against cell oxidation. This also corroborates its use in folk medicine as one of the first line treatment methods upon consumption of toxic or poisonous substances. However, since the data reported from this study were generated for short-term treatment with palm oil and coconut water, it is recommended that long term animal studies be carried out to evaluate the effects of palm oil and coconut liquor on these biomarkers as well as the biochemical mechanisms involving xenobotic enzymes.

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REFERENCES

Anurag P. and Rajamohan, T. (2003). Beneficial effects of tender coconut water against isoproterenol induced toxicity on heart mitochondrial activities in rats. *Ind J BiochemBiophys*40: 278-280.

Barclay, E. (2011).Coconut Water to the Rescue? Parsing the Medical Claims. *NPR*. 1:2-16

Bazzano, L.A.; He, J.; Ogden, L.G.; Loria, C.M.; Vupputuri, S.; Myers, L. and Whelton, P.K. (2002). Fruit and vegetable intake and risk of cardiovascular disease in US adults. The first national health and nutrition examination survey epidemiologic follow-up study. *Am. J. Clin. Nutr.76*:93–99.

Bergmeyer, H. U., Horder, M. and Rej, R. (1985). Approved recommendation of IFCC methods for the measurement of catalytic concentration of enzymes part 3. IFCC method for

alanine aminotransferase. *Eur. J. Clin. Chem. Clin. Biochem*., 24: 418 – 489.

Bhagya, D., Prema, L. and Rajamohan,T. (2010). Beneficial effects of tender coconut water on blood pressure and lipid levels in experimental hypertension. *J Cell Tissue Res 10*: 2139-2144.

Borzelleca, J.F., E.C. Clarke, and L.W. Condie Jr., (1989). "Short-term Toxicity (1 and 10 days) of Cadmium Chloride in Male and Female Rats: Gavage and Drinking Water", *J. Amer. Coll. Toxicol*., 8: 377-404.

Campbell-Falck, D., Thomas, T., Falck, T. M., Tutuo, N. and Clem, K. (2000).The intravenous ues of coconut water. *Am J. Emerg Med.* 18(1): 108 -111.

Depeint, F.; Bruce, W.R.; Shangari, N.; Mehta, R. and O'Brien, P.J. (2006) Mitochondrial function and toxicity: Role of B vitamins on the one-carbon transfer pathways. *Chem. Biol. Interact. 163*:113–132.

Drotman, R. B. and Lawhorn, G. T. (1978). Serum enzymes and indicators of chemically induced liver damage. *Drug chem..Toxicol*., 1: 163 -171.

Elinder, C. G., (1985)."Normal Values for Cadmium in Human Tissues, Blood, and Urine in Different Countries", in: Cadmium and Health: A Toxicological and Epidemiological Appraisal, Vol. 1, L. Friberg, C-G. Elinder, T. Kjellstrom, and G.F. Nordberg (eds.), CRC Press, Boca Raton, FL, pp. 81-102.

Institute of Medicine (IOM) (2000). *Dietary Reference Intakes for Calcium, Phosphorus, Magnesium,Vitamin D, and Fluoride*; National Academy Press: Washington, DC, USA .

Kamat, J. P. and Devasagayam, T. P. (1995). Tocotrienols from palm oil as potent inhibitor of lipid peroxidation and protein oxidation.*NeurosciLett*. 195(3):179 - 182.

Kaplowitz, N. (2001). Drug-induced liver disorders: Implication for drug development and regulation. Drug Safety, 24: 483 – 490.

Kende, H. and Zeevaart. J. (1997) The five “Classical” plant hormones. *Plant Cell*, *9*: 1197–1210.

Khandelwal, S., Agnihotri, N. and Tandon, S. K. (1991). Biochemical response to cadmium: Dose-time effect. *Biol. Trace Elem. Res*. 29: 157 – 164.

Klauke, R., E. Schmidt and Lorentz, K. (1993). Recommendation for carrying out standard ECCLS procedures (1988) for the catalytic concentrations of creatine kinase, aspartate aminotransferase, alanine aminotransferase and gamma-glutamyltransferase at 37ºC. Standardization committee of the German society for clinical chemistry, enzyme working Group of the German society for clinical chemistry. *Eur. J. Clin. Chem. Clin. Biochem*., 31: 901 – 909.

Kobayashi, H.; Morisaki, N.; Tago, Y.; Hashimoto, Y.; Iwasaki, S.; Kawachi, E.; Nagata, R. and Shudo, K. (1997). Structural identification of a major cytokinin in coconut milk as 14-*O*-(3-*O*-[β-Dgalactopyranosyl-(1-->2)-α-D-galactopyranosyl-(1-->3)-α-L-arabinofuranosyl]-4-O-(α-L-arabinofuranosyl)- β-D-galactopyranosyl)-*trans*-zeatin riboside. *Chem. Pharm. Bull. 45*: 260 –264.

Latinwo, L. M., Ikediobi, C.O., Singh, N. P., Sponholtz, G., Fasanya, C. and Riley, L. (1997). Comparative studies of in vivo genotoxic effects of cadmium chloride in rat brain, kidney and liver cells.*Cell MolBiol* (Noisy-le-grand) 43(2): 203 – 210.

Lowry, O. H., Rose Brough, N.J. and Randal, R. J. (1951). Protein measurements with the folin phenol reagent.*J BiolChem*193: 265-275.

Ma, Z.; Ge, L.; Lee, A.S.Y.; Yong, J.W.H.; Tan, S.N. and Ong, E.S. (2008) Simultaneous analysis of different classes of phytohormones in coconut (*Cocos nucifera* L.) water using high-performance liquid chromatography and liquid chromatography-tandem mass spectrometry after solid-phase extraction, *Anal. Chim. Acta 610*: 274–281.

Manca, D, Ricard, A. C., Trottier, B. and Chevalier, G. (1991).Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride.*Toxicology* 67 (3): 303 – 323.

Mann, D. (2012). Can Heavy Metal in Foods, Cosmetics Spur Breast Cancer Spread? HealthDay. Presentation, Experimental Biology 2012 meeting, San Diego

McDowell, L. R. (1992). Minerials in Animals and Human Nutrition.*Academic Press, San Diego, CA., USA* 30: 111-117.

Moss, D. W. and Butterworth, P. J. (1974). Enzymology and Medicine, Pitman Medicine, London, pp:139.

Noël, L., Guérin, T. and Kolf-Clauw, M. (2004). Subchronic dietary exposure of rats to cadmium alters the metabolism of metals essential to bone health. *FoodChem*. *Toxicol*. 42: 1203–1210

Okorie, E. O.; Young Bae, J.; Jun-Ho, L.; Seunghyung, L.; Gun-Hyun, P.; Mahmoud, M. and Sungchul, C. B. (2014).Effects of Different Dietary Cadmium Levels on Growth and Tissue Cadmium Content in Juvenile Parrotfish, *Oplegnathus fasciatus* . *Asian Australas J. Anim. Sci* 27 (1): 62- 68.

Oluba, O., Onyeneke, C., Ojieh, G., Eidangbe, G., Orole, R. (2008).Effects of Palm Oil Supplementation on Lipid Peroxidation and Glutathione Peroxidase Activity in Cholesterol – Fed Rats.*The Internet J. Cardiovasc Res*.6 (1). 19 -21.

Oyagbemi, A. A. and Odetola, A. A. (2010). Hepatoprotective Effects of Ethanolic Extract of *Cnidoscolusaconitifolius* on Paracetamol-Induced Hepatic Damage in Rats. *Pakistan Journal of Biological Sciences* 13(4): 164-169.

Paniappan, S. (2002).The Mystery Behind Coconut Water.*TheHindu*. 2(15):221- 229.

Prathapan, A. and Rajamohan , T. (2011). Antioxidant and antithrombotic activity of tender coconut water in experimental myocardial infarction. *J Food Biochem 35*: 1501-1507.

Preetha, P. P., Devi, V. G. and Rajamohan, T. (2013). Comparative effects of mature coconut water (*Cocos nucifera*) and glibenclamide on some biochemical parameters in alloxan induced diabetic rats. *Rev. bras. farmacogn.* 23 (3): 156 - 163

Preetha, P.P., Devi, V. G. and Rajamohan, T. (2012). Hypoglycemic and antioxidant potential of coconut water in experimental diabetes. *Food Funct 3*: 753-757.

Pummer, S., Heil, P. W. and Petroianu, G. (2001).Influence of coconut water on homeostasis. *Am J.Emerg Med* 19: 287 – 289.

Reeves, J. B. and Weihrauch, J. L. (1979).Consumer and Food Economics Institute. Composition of foods: fats and oils. *Agriculture handbook* 1: 8 - 4.

Rhman, N. N. A., Bakhiet, A. O. and Adam, S. E. I. (2011). Toxic Effects of Various Dietary Levels of combined Cadmium chloride and Zinc chloride on Male Wistar Rats. *J. Pharmcol. Toxicol*. 6(1):76 – 81.

Riboli, E. and Norat, T. (2003) Epidemiologic evidence of the protective effect of fruit and vegetables on cancer risk, *Am. J. Clin. Nutr. 78*: 559–569.

Saskia R., Gudrun de Boeck , Hilde De Cock, Ronny, B. and Lieven, B. (2014). Accumulation and detoxification of metals and arsenic in tissues of cattle (*Bostaurus*), and the risks for human consumption. *Sci. Total Environ.*466–467:175 –184

Sen, C. K., Khanna, S., Rink, C., and Roy, S. (2007). Tocotrienol: the emerging face of natural Vit E. *Vitamin Horn* 76: 203- 261.

Sen, C. K., Rink, C., and Khanna, S. (2010). Palm Oil–Derived Natural Vitamin E α-Tocotrienol in Brain Health and Disease. *J Am Coll Nutr.* 29(3 Suppl): 314S–323S.

Shenkin, A. (2006) The key role of micronutrients. *Clinical Nutr. 25* :1–13.

Sirajudeen, K. N. S. (2006): Toxicity Studies of *Phyllanthus* Species. Traditional herbal medicines for modern times. Ramadasan, K. and Harikumar, K. B. (eds). CRC Press, USA. Pp 279 – 288.

Tietz, N. W. and Shuey, D. F. (1986). Reference intervals for alkaline phosphatase activity determined by the IFCC and AACC reference methods *Clin. Chem*., 32: 1593 – 1594.

Tucker, G.A. and Roberts, J.A. (2000) Plant Hormone Protocols; Humana Press Inc.: Totowa, NJ, USA.

Valdes, B. P. and Ziobro, G. C. (2000). Regulatory Action criteria for Filth and other Extraneous materials IV. Visual Detection of Hair in Food. *Regulatory Toxicology and Phamacology (*Academic Press) 32 (1): 73 – 77.

Vallec, B. L. and Ulmer, D. D. (1972). Biochemical effects of mercury, cadmium and lead. *Annu. Rev. Biochem.* 41: 91-128.

WHO (1992). Cadmium. International programme on chemical safety. *Environmental Health Criteria*, 16: 134 – 135.

Wu, Y. and Hu, B. (2009) Simultaneous determination of several phytohormones in natural coconut juice by hollow fiber-based liquid-liquid-liquid microextraction-high performance liquid chromatography, *J. Chromatogr. A,* 1216 :657–7663.

Yong, J. W. H., Ge, L., Ng, Y. F. and Tan, S. N. (2009). The Chemical Composition and Biological Properties of Coconut (*Cocos nucifera* L.) Water. *Molecules 14*:5144-5164.