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RENOPROTECTIVE PROPERTY OF Chrysophyllum albidum EXTRACT AGAINST Pb^{2+}-INDUCED NEPHROTOXICITY AND OXIDATIVE STRESS IN WISTAR RATS

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ABSTRACT

Nephrotoxicity and myriads of renal disorders are currently on the increase worldwide. This poses a great threat to the medical sector globally thereby necessitating the continued search for bioactive agents with reliable renoprotective property. In this study a flavonoid-rich-extract (FRE) of *Chrysophyllum albidum* pulp was assessed for protective property against lead-induced nephrotoxicity in wistar rats. The animals were grouped into four A, B, C and D and were kept under normal condition for 2-weeks. The positive control group B was administered 2.5mg/kg lead acetate while negative control group A received distilled water only. Animals in group C and D simultaneously (1:1) received 2.5mg/kg lead acetate with 150mg/kg FRE and 2.5mg/kg lead acetate with 250mg/kg FRE respectively. Results revealed that lead caused a significant ($P<0.05$) increase in level of thiobarbituric acid reactive substances in the kidney which was restored in group treated with FRE. Lead also caused significant increase in serum levels of other evaluated kidney indices compared with the control. However, a dose of 250mg/kg FRE brought about a significant ($P<0.05$) reduction in the levels of these indices while at 150mg/kg there was no significant effect on the parameters assessed compared with the lead acetate intoxicated group. The results obtained from the *in vitro* and *in vivo* studies demonstrated the renoprotective potential of *Chrysophyllum albidum* pulp flavonoid rich extract.

Key words: Lead–Chrysophyllum albidum–nephrotoxicity–oxidative stress.

1 INTRODUCTION

The global importance of medicinal plant resources is evident in the exponential increase in the number of people seeking therapeutic remedies especially towards the last half of the twentieth century for anticancer compounds mainly from tissue cultures of medicinal plants [1] . The mission statements of WHO on health improvement buttress this in the initiatives by international organizations. The World Bank recognized the role of medicinal plants in the health care delivery system and recommended that proven traditional herbal remedies should be incorporated within the national drug policies of all nations of the world [2] . Medicinal plants play a pivotal role in the health care of ancient and modern culture for example the Indian system of health care delivery always rely on plant based drugs to treat human ailments because they contain the components of therapeutic value [3] . Lead and other related heavy metals are generally referred to as environmental toxicants [4] . Exposure to lead especially through consumption of contaminated well water has been associated with skin lesion, liver damage and chromosome aberrations [5] as well as certain forms of cancer and neurological disorders [6] . This research was designed

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to investigate the renoprotective properties of flavonoid rich extract of *Chrysophyllum albidum* pulp against lead-induced nephrotoxicity in wistar rats

2 MATERIALS AND METHODS

Plant Extract

The *Chrysophyllum albidum* pulps were air dried for 28 days at room temperature. The air dried pulps were ground to fine powder. 500g sample of the fine powder was soaked in 2000ml of distilled water for 72hrs, filtered and freeze dried to obtain the extract and was kept in freezer at 4^0C for further studies.

Chemicals and Reagents

Lead acetate [$(CH_3COO)_2Pb.3H_20$ mol. wt. 379.33, 99.999%, CAS No. 6080-56-4; Aldrich Chemical Co. Inc. (U.S.A)] were dissolved in distilled water and 2-3 drops of acetic acid was used to dissolve the lead acetate precipitate. They were administered at a dose of 2.5mg/kg body weight corresponding to $^1/_{10th}$ of approximate environmental human daily exposure level [7] . All other reagents and chemicals were of analytical grade and were obtained from Sigma Chemical Co.St. Lous, MO, USA.

Biochemical parameters

Selected biochemical parameters for the kidney indices were assayed using the metabolic panel plus reagent discs on an automated chemistry analyzer (Piccolo Blood Chemistry Analyzer, Abaxis, Union City, CA, USA). The parameters assayed were glucose, creatinine, blood urea nitrogen, alkaline phosphatase (ALP), total protein, total bilirubin, lactate dehydrogenase (LDH), and albumin.

Reduced Glutathione (GSH)

0.2ml *Chrysophyllum albidum* extract was added to 1.8ml of distilled water and 3ml of sulphosalicylic acid. The mixture was centrifuged at 3000 xg for 4min and 0.5ml of the supernatant was added to 4.5ml of Ellman's reagent. A blank was prepared with 0.5ml of dilute precipitating agent and 4ml of phosphate buffer with 0.5ml of Ellman's reagent. The absorbance was taken within 30mins of colour development at 412nm against blank and the concentration of GSH was extrapolated from the GSH standard curve using method by Beutler et al., (1963).

Glutathione Peroxidase (GPx) Assay

500 l *Chrysophyllum albidum* was added to reaction mixture containing 500 l phosphate buffer pH 7.4, 100 l sodium azide, 200 l GSH, and 100 l hydrogen peroxide, after which 600 l distilled water was added and mixed thoroughly. The whole reaction mixture was incubated at 37^0C for 3mins after which 0.5ml of TCA was added and later centrifuged at 3000rpm for 5mins. K_2HPO_4 (2ml) and 1ml of DNTB was added to 1ml of each of the supernatants and the absorbance was read at 412nm against blank. The GPx activity in the sample was determined according to the method described by Rotruck et al., (1973).

Superoxide-dismutase (SOD) Assay

0.2ml *Chrysophyllum albidum* was added to 2.5ml of 0.05M carbonate buffer of pH 10.2 to equilibrate in the spectrophotometer. The reaction was initiated by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The reference cuvette contained 2.5ml buffer, 0.3ml of adrenaline and 0.2ml distilled water. The increase in absorbance at 480nm was monitored every 30secs for 150secs.according to the method described by [8] .

Catalase (CAT) Assay

70 l *Chrysophyllum albidum* was mixed with 920 l sodium phosphate buffer containing 0.1mM EDTA at pH 7.0. The reaction was started by adding 10 l of hydrogen peroxide. The decrease in hydrogen peroxide concentration was taken by reading the absorbance at 240nm at 10secs intervals for 180secs. Aebi, (1974) (14) method was used to determine the catalase activity:

Lipid Peroxidation Assay

The rats were decapitated and the kidneys excised, homogenized at approximately 1200rev/min in Teflon glass homogenizer. The homogenate was centrifuged and the supernatant was used for the assay(Belle et al, 2004). The TBARS assay was carried out using (Ohkawa, et al, 1979) and the thiobarbituric acid species produced were measured at 532nm.

3 RESULTS

Kidney Indices

Table 1.

Parameter	A	B	C	D
Urea Nitrogen (mg/dl)	1.31+1.10a	6.02+3.03a	6.32+0.95a	0.81+0.42a
Creatinine (mg/dl)	0.07+0.01a	0.08+0.01a	8.09+0.02a	1.08+0.01a
Albumin (g/dl)	2.20+1.25a	4.73+1.31a	4.83+0.19a	2.44+0.30a
Total Bilirubin (mg/dl)	0.54+0.01a	7.56+0.01a	5.55+0.19a	3.54+0.02a
Total Protein (g/dl)	7.90+0.50a	9.25+9.20a	8.20+0.029	8.42+0.12a
Glucose (mg/dl)	13.90+8.51a	7.76+4.16b	37.33+10.07	23.16+5.60b
Alkaline Phosphatase (U/L)	117.24+4.01a	30.33+11.24b	35.32+6.04b	21.45+20.27a
Lactate dehydrogenase (U/L)	53.11+5.68a	2.67+8.49b	0.56+18.32b	7.34+27.38b

Resultsare expressed as means + standard deviation. Values along the horizontalrow with different superscripts indicate significant difference at (P <0.05).

4 DISCUSSION

The results from this study indicate synergistic effect of the extract on the defensive antioxidant enzymes (GSH, GPx, SOD and CAT) with concomitant increase in their activities corresponding to increase in extract concentration as shown in Figure 1, 2, 3, and 4. This effect could be attributed to the presence of flavonoids, selenium and zinc in the extract

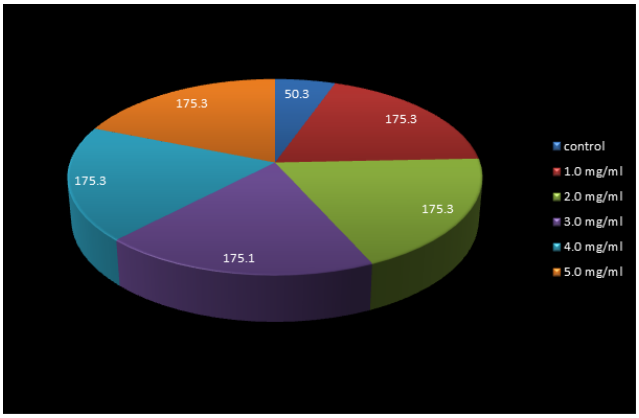

Figure 1. Effect ofCrysophyllum albidum aqueous extract on reduced glutathione activity

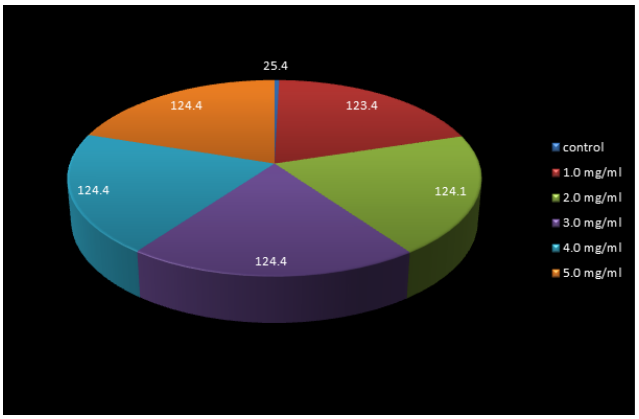

Figure 2. Effect ofChrysophyllum albidum on glutathione peroxidase activity

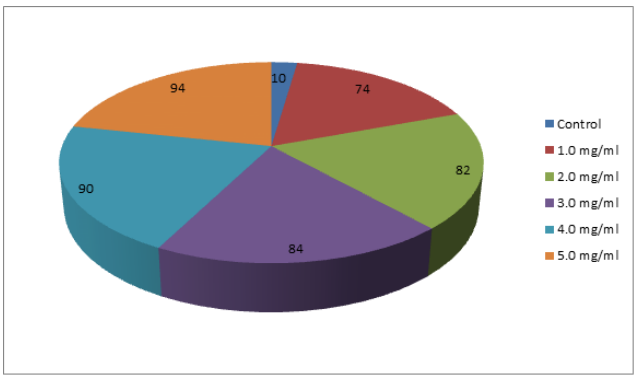

Figure 3. Effect of Chrysophyllumalbidum on superoxide dismutase activity

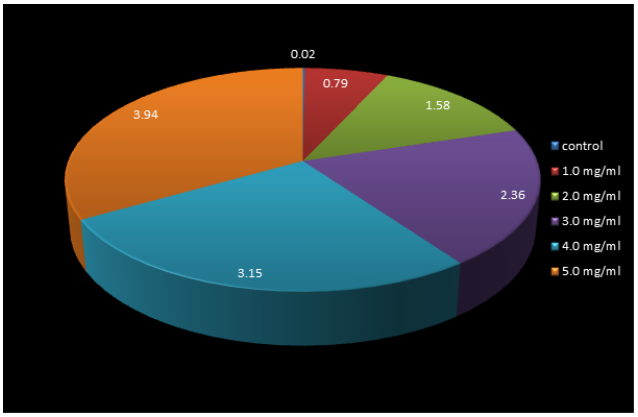

Figure 4. Effect of Chrysophyllum albidum aqueous extract on catalase activity

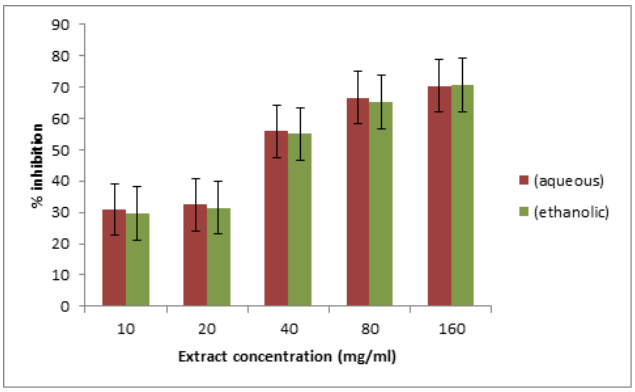

Figure 5. Inhibitory effect of Chrysophyllum Albidum on Pb^{2+}-inducedlipid peroxidation in rat kidney

which generally activate the antioxidant enzymes [9] , thus prevent rise in concentration of free radicals that cause cellular assault. In addition, the results obtained from lipid peroxidation assay in vitro showed remarkable increase in levels of thiobarbituric acid reactive species on administration of Pb^{2+} as the proxidant. The findings that Fe^{2+} caused a significant increase in malondialdehyde and thiobarbituric acid reactive species MDA/TBARS in brain and kidney [10] agreed with this study. However, the elevated thiobarbituric acid reactive species was sharply decreased with the administration of the extract of Chrysophyllum albidum pulp. Figure 5 above shows that ethanolic extract of Chrysophyllum albidum exhibited higher inhibitory potential (70%) against Pb^{2+} induced lipid peroxidation in the rat kidney at 80mg/ml extract concentration compared to aqueous extract at (67%) inhibitory potential at same extract concentration. Lead is an oxidant capable of inducing oxidative stress in the liver and brain cells leading to cancer and neuronal disorders such as cerebral edema [11] . The inhibitory potential demonstrated by Chrysophyllum albidum pulp extract in this study could be attributed to its inherent active phytochemicals that promote its antioxidant activities. Besides, the reduction in the concentration of ALP, LDH, albumin, urea nitrogen, bilirubin and crea-

tinine at varying doses of 150mg/kg and 250mg/kg Chrysophyllum albidum was significant ($P<0.05$) in group D animals fed with 250mg/kg compared to control. This could be attributed to the activity of inherent antioxidant properties of the extract which suggests possible renoprotective potential of the extract since renal damage is often assessed by increase in serum ALP, urea nitrogen and LDH [12] . On the contrary, the significant ($P<0.05$) increase in ALP, and LDH observed in the kidney of group B animals fed lead acetate could be due to increase in functional activities leading to an induction of these enzymes probably due to de novo synthesis [13] . Also, the significant ($P<0.05$) increase in levels of creatinine, albumin and total bilirubin in group B and C animals was as a result of lead toxicity in the systemic system of the animals [14] . However, the significant ($P<0.05$) increase in glucose level (32.76) observed in group B animals compared to control (13.90) and which was gradually restored in group D animals fed with 250mg/kg of Chrysophyllum albidum pulp extract, may be due to oxidative effect of lead on liver beta cells which may further impair the secretion of insulin [15] . However, there was no significant effect on the total protein of the test rats compared to control .

REFERENCES

[1] G, Roja R, S P. Anticancer compounds from tissue cultures of medicinal plants; global importance of medicinal plant resources. J Herbs Med Plants. 2000;7:71–102.

[2] O WH. Guides on proven traditional medicine and administration. Environmental Health Criteria. 2001;58:401–405.

[3] Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E. Effects of flavonoids, tannins and related polyphenols on superoxide anion radicals. and. 1989;1(1):2–37.

[4] Elias RW. Effects of lead exposure, dietary and environmental lead in human health. Elsevier Amsterdam New-York-Oxford. 1985;p. 79–107.

[5] S O, Tugbobo O, I O. Determination of lead concentration in water samples and modification of its mutagenic effect by crude garlic extract. Bull Environmental Science. 2009;2009:115–119.

[6] Valentine JL. Review of health assessment for Canada/US population exposed to arsenic in drinking water. Geochemistry and Health Journals. 1994;16:139–152.

[7] Preston RJ, Dean BS, Galloway S, Shelby M. Mammalian in vivo cytogenetic assay; analysis of chromosomal aberration in bone marrow cells. Mutat Res. 1987;189:157–165.

[8] P H, Misra F, I. The role of superoxide anion in the autooxidation of epinephrine and simple assay for superoxide dismutase. J Biol Chem. 1972;247(12):3170–3175.

[9] K S, Das N, C R. Superoxide dismutase, glutathione peroxidase, catalase and lipid peroxidation of normal and sickled erythrocytes. Br J. 1980;haematol. 44: 87 – 92.

[10] Oboh G, L R, Puntel R, T JB. Hot pepper (Capsicum annuum) prevents Fe2+ induced lipid peroxidation in brain in vitro. Food chemistry. 2007;102:178–185.

[11] Needleman H. Lead-induced cerebral edema. Food Chem. Toxicol. 1993;47(9):743–749.

[12] Menin BF, Sherins R, Mann T. Renal damage and effect on lactate dehydrogenase. Free Radical Biol Med. 2001;5:368–374.

[13] Yakubu MT, Bukoye B, T A, Oladiji A, A M. Toxicological implications of aqueous extract of Bambusa vulgaris leaves in pregnant Dutch rabbits. Human & Exp. Toxicol. 2009;28(9):591. 598.

[14] Tugbobo OS, Adeniyi OA, Oyewusi HA. Assessment of antioxidant and toxicological effects of Allium sativum extract in rat liver in vitro. Der Pharmacia Lettre. 2016;2016(8):274–279.

[15] Sato Y, Hotta N, Sakamoto N, Matsuoka S, Ohisi N, Yagi K. Lipid peroxide level in plasma of diabetic patient. Biochem Med Metab Biol. 1979;21:104–107.

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