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Hemoprotective and nephroprotective potentials of aqueous extract of *Jussiaea nervosa* leaf in cadmium exposed albino rats.

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Abstract: *In this study, the hemoprotective and nephroprotective potentials of aqueous extract of Jussiaea nervosa leaves on cadmium-exposed rats were evaluated. In the first experiment, 24 Wister albino rats divided into 6 groups: A-F of 4 rats per group were used for the evaluation of Cd toxicity. While group A rats received water and feed only and served as negative control (NC), groups B-F received in addition to water and feed, 1, 2, 4, 8 & 12 mg/kg body weight of Cd, respectively for 6 weeks. Based on the significant effect of Cd at 12mg/kg body weight in the first experiment, additional group (G), exposed to 12mg/kg body weight of Cd was established for the assessment of the hemoprotective and nephroprotective potentials of J. nervosa in Cd-exposed rats. This group (n = 12) was subdivided into four subgroups (G1, G2, G3 and G4) of 3 rats each. Subgroup G1 was maintained on normal feed and water and served as the positive control (PC) while G2, G3 and G4 were given 20, 50 and 100 mg/kg doses of aqueous Jussiaea nervosa extract, respectively. All feedings and treatment also lasted for six weeks. At the end of the experiment, the animals were sacrificed and blood and kidney samples were collected for laboratory analyses. Biochemical and histological analyses were done using standard laboratory techniques. Exposure to cadmium was observed to cause significant ($p < 0.05$) decreases in packed cell volume (PCV), hemoglobin concentration (HBC), and total white blood cell count (TWBC) and increases in plasma urea, creatinine & electrolytes. However, administration of aqueous extract of J. nervosa ameliorated these Cd-induced effects in a dose-related manner, with higher doses (50 mg/l & 100 mg/l) almost restoring the parameters to the levels in non-exposed rats. Additionally, the Cd-induced changes in the histology of the kidneys were almost restored by administration of Jussiaea nervosa extract. We conclude that J. nervosa may possess haemoprotective and nephroprotective potentials and its regular consumption may protect against cadmium toxicity.*

Key words: *Jussiaea nervosa, Cd-intoxication, hematoprotection, nephroprotection*

I. Introduction

Cadmium (Cd) has been classified as a category one carcinogen by International Agency for Research on Cancer. It causes cancer of the lungs, pancrease, prostate and kidney[1] .The general population is facing an increasing risk of Cd exposure and the attendant health hazards associated with it are of immense concern. One of the main sources of Cd exposure is cigarette smoke[2]. In addition, Cd is found in ambient air in occupationally exposed situations and in paints [3].

In recent times, the use of traditional medicine is increasingly gaining popularity in both the developed and developing Worlds. It has been estimated that about 80% of the people in developing countries rely on traditional medicine for their health care [4]. In Nigeria, the effectiveness of some indigenous plants in the treatment of various ailments is no longer in doubt [5].

Jussiaea nervosa is one of the herbal plants popularly used for treating various diseases. Its uses include poison antidote, treatment of alcohol intoxication, diarrhoea, dysentery, vomiting, just to mention a few [6]. The parts of the plant usually used for treatment of diseases are the leaves and soft stems. *Jussiaea nervosa* is of the family of *Onagracea*. The common local names include *arira mmili* (Igbo), *sha shatau* (Hausa), and *ewuru odo* (Yoruba).

Considering the ease with which humans are exposed to Cd, both at home (terrestrial foods, drinking water, cigarette), work places and the abundance of *Jussiaea nervosa* in our environment, it has become pertinent to scientifically evaluate whether the consumption of the leaf extract will protect against Cd toxicity.

The aim of the present study is to determine the hemoprotective and nephroprotective potentials of aqueous leaf extract of *Jussiaea nervosa* in Cd-exposed rats.

II. Materials and Methods

2.1. Preparation of extract

The leaves of *Jussiaea nervosa* was collected from a farm in a swampy area of phase 6, Trans-Ekulu, Enugu East Local Government Area of Enugu State, Nigeria. The plant was identified and authenticated by Prof. J.C. Okafor, a Consultant Agro forester and Taxonomist, University of Nigeria, Nsukka. The leaves were washed and sun-dried to constant weight and ground into powder with a manual grinder. The resulting powder was soaked in distilled water and boiled on slow heat for about two hours.

The preparation was then suction-filtered and the process repeated until all the soluble compounds had been extracted. This was judged by loss of color of the filtrate. The extract was concentrated to dryness at 60 ^{o}C using electric oven. It was carefully evaporated to dryness on water bath at 40 ^{0}C [7] and the extract stored in a refrigerator until use.

2.2. Animals and treatment

Male Wister albino rats (n = 24) weighing 105-162 g purchased from animal house of the Department of Pharmacy, University of Nigeria, Nsukka were randomly assigned into six (6) groups (A-F) of five (4) rats per group. The animals were allowed free access to feed and water *ad libitu* for a period of one week to allow them acclimatize. All the rats received human care in accordance with the National Institute of Health guidelines for the care and use of laboratory animals [8].

Group A rats served as negative control (NC) and were given only water and feeds. Groups B, C, D, E and F were exposed to Cd at concentrations of 1, 2, 4, 8 and 12 mg/kg of body weight respectively, for six weeks. They were also fed with water and feeds *ad libitum*. The effect of Cadmium observed in rats exposed to 12 mg/kg body weight was found to be most significant and this informed the use of 12 mg/kg Cd-concentration in subsequent study involving herbal intervention with *Jussiaea nervosa* extracts. In this segment of the study, additional group (G) was established. This group containing 12 rats was sub-divided into 4: G_1, G_2, G_3 and G_4 sub-groups, with each sub-group containing 3 rats. While sub-group G_1 rats were given water and feed only and served as positive controls (PC), sub-groups G_2, G_3, and G_4 rats were given 20, 50 and 100 mg/kg body weight of *Jussiaea nervosa* extract in addition to water and feed, respectively.

All feeding and treatments were for six weeks duration. At the end of the experiment, the rats were anaesthetized in a chloroform saturated chamber and sacrificed. Blood samples were taken from each of the rats for haematological and biochemical analysis, while the kidneys were excised for histopathological analysis.

2.3. Sample analyses

Hematological parameters {packed cell volume (PCV), hemoglobin concentration (HBC), total white blood cell count (TWBC)} were analyzed as described by Dacie and Lewis [9], plasma urea was determined by the method described by Jung *et al.*[10] and creatinine estimation was done by method originally described by Benedict and Behie [11] and reevaluated by Stevens *et al.* [12], plasma potassium (K^+) and sodium (Na^+) were determined by flame atomic absorption spectrophotometer, bicarbonate ($HCO3^-$) was determined by titration in accordance with the method described by Van Slyke [13], Serum chloride (Cl^-) was estimated by the mercuric nitrate colorimetric method described by Skeggs and Hochstrasser [14].

The histopathological analysis of the kidney was carried out using standard methods according to Talib and Khurana [15].

2.4. Statistical analysis

Results were expressed as mean ± standard deviation. Comparison of parameters among groups was done by one-way analysis of variance (ANOVA) with statistical significance achieved at $p < 0.05$.

III. Results

Table1: Effects of Cadmium exposure on hematological parameters of Cadmium exposed albino rat

Rat groups	*Cadmium dose (mg/Kg body weight)*	*PCV (%)*	*HBC (g/dl)*	*TWBC ($x10^9$/l)*
A	0.0	52.66 ± 1.35^{a}	17.37 ± 0.36^{a}	6.40 ± 0.87^{a}
B	1.0	48.33 ± 0.96^{a}	14.60 ± 0.29^{a}	5.60 ± 0.66^{a}
C	2.0	38.66 ± 1.35^{b}	12.90 ± 0.58^{b}	5.00 ± 0.64^{a}
D	4.0	35.60 ± 2.50^{b}	11.73 ± 0.68^{b}	4.7 ± 0.58^{b}
E	8.0	32.00 ± 2.87^{b}	9.90 ± 0.12^{b}	4.00 ± 0.18^{b}
F	12.0	28.60 ± 1.35^{bc}	6.36 ± 1.35^{bc}	3.10 ± 1.12^{bc}

PCV: Packed cell volume; **HBC**: Hemoglobin concentration; **TWBC**: Total white blood cell count. Values are expressed as mean ± s. d. Values with different superscripts are significantly different.

From table 1, exposure to cadmium was observed to have adverse effect on the hematological parameters of the exposed rats with packed cell volume, hemoglobin concentration and total white blood cell count significantly decreasing with increasing doses of cadmium. However, at cadmium dose of 12mg/Kg body weight of the rats, PCV, HBC and TWBC were found to decrease by 45.7 %, 63.4 % and 51.6 %, respectively.

Table 2: Effects of aqueous extract of *Jussiaea nervosa* leaf on hematological parameters of cadmium exposed albino rats

Rat groups	*Extract dose (g/l)*	*PCV (%)*	*HBC (g/dl)*	*TWBC ($x10^9$/l)*
A	0.0 (NC)	52.66 ± 1.35^a	17.37 ± 0.36^a	6.40 ± 0.87
G_1	0.0 (PC)	28.60 ± 1.35^b	6.36 ± 1.35^b	3.90 ± 0.12^b
G_2	20.0	49.00 ± 0.57^a	15.58 ± 1.20^a	7.23 ± 0.73^a
G_3	50.0	52.66 ± 1.35^a	17.66 ± 0.12^a	8.80 ± 0.11^a
G_4	100.0	55.33 ± 3.43^a	19.33 ± 0.10^a	9.10 ± 0.64^a

NC: Negative control; PC: Positive control; PCV: Packed cell volume; HBC: Hemoglobin concentration; TWBC: Total white blood cell count. Values are expressed as mean ± s. d. Values with different superscripts are significantly different.

Table 2 shows the effects of *J. nervosa* on the hematological parameters of cadmium exposed rats. Although hematological parameters in cadmium exposure rats were found to be significantly ($p < 0.05$) lower in comparison to the non-exposed rats, administration of aqueous extract of *J. nervosa* showed ameliorative effects. These ameliorative effects were observed to be dose dependent, with higher doses (50mg/l & 100mg/l) in addition to restoring the parameters to the levels in non exposed rats, tends to cause increases in these parameters above the values found in non- exposed group.

Table 3: Effects of cadmium exposure on plasma urea, creatinine and electrolytes in cadmium exposed albino rats

Rat groups	Cd dose	Urea	Creatinine	Electrolytes			
				K^+	Na^+	Cl^-	HCo_3^-
A	0	5.38 ± 0.42^a	65.75 ± 0.14^a	3.58 ± 0.01^a	134.18 ± 0.22^a	101.50 ± 0.52^a	25.43 ± 1.49^a
B	1	7.07 ± 0.10^a	89.45 ± 1.18^b	5.25 ± 0.17^a	135.53 ± 0.21^a	103.30 ± 2.14^a	26.45 ± 0.32^a
C	2	8.12 ± 0.30^a	94.25 ± 3.06^b	5.07 ± 0.37^a	137.06 ± 0.09^a	105.93 ± 0.04^a	25.63 ± 0.39^a
D	4	10.36 ± 0.28^b	95.27 ± 2.84^b	5.34 ± 0.25^a	139.18 ± 0.09^a	107.78 ± 0.64^a	28.59 ± 0.35^a
E	8	12.28 ± 0.43^b	108.08 ± 1.74^c	5.80 ± 0.57^a	141.07 ± 0.09^b	109.66 ± 0.27^a	29.23 ± 0.52^a
F	12	16.38 ± 0.09^{bc}	114.12 ± 0.69^c	6.10 ± 0.75^b	145.20 ± 0.06^b	110.44 ± 0.18^b	31.49 ± 0.96^b

The effects of exposure to cadmium on plasma urea, creatinine and electrolytes were shown in table 3. Exposure to cadmium was observed to cause impairment in renal functions in dose dependent manner, with higher doses having more severe effects as demonstrated by higher elevations in plasma urea and creatinine at higher doses. Additionally, cadmium toxicity was observed to affect plasma urea and creatinine (which almost tripled and doubled respectively at Cd dose of 12mg/Kg body weight) more than the electrolytes, except for potassium which almost doubled at the same cadmium dosage.

Table 4: Effects of aqueous extract of *Jussiaea nervosa* on plasma urea, creatinine and electrolytes in cadmium exposed albino rats

Rat groups	Cd dose	Urea	Creatinine	Electrolytes			
				K^+	Na^+	Cl^-	HCo_3^-
A	NC	5.38 ± 0.42^a	65.75 ± 0.14^a	3.58 ± 0.01^a	134.18 ± 0.22^a	101.50 ± 0.52^a	25.43 ± 1.49^a
G_1	PC	16.60 ± 0.09^b	114.12 ± 0.62^b	6.10 ± 0.75^b	145.20 ± 0.16^b	110.44 ± 0.18^b	31.49 ± 0.94^b
G_2	20	16.00 ± 0.29^b	112.17 ± 3.07^b	5.51 ± 0.05^b	143.90 ± 0.12^b	108.47 ± 0.04^b	29.14 ± 0.94^a

G_3	50	15.50±0.25[b]	110.13±3.97[b]	5.05±0.21[b]	140.90±0.18[b]	105.40±0.4[a]	28.53±0.49[a]
G_4	100	15.00±0.07[b]	108.80±2.25[b]	4.00±0.20[a]	130.05±0.20[a]	103.05±0.20[a]	26.08±0.59[a]

Values are expressed as mean ± s. d. Values with different superscripts are significantly different.

Although administration of aqueous extract of *J. nervosa* at higher doses improved the electrolyte status in cadmium-induced renal impairment, with plasma K^+, Na^+, Cl^-, and HCO_3^-, which were elevated in untreated rats almost returned to levels found in non-exposed rats, at *J. nervosa* dosage of 12mg/Kg body weight, the effects on plasma urea and creatinine were non-significant (Table 4).

Histological analysis showed high degree of damage to the kidney characterised by severe congestion and infiltration of inflammatory cells, widespread injury on glomeruli, haemorrhage, pkynosis and excessive necrosis in Cd-exposed rats (Figure 2) in comparison to the control (Figure 1). However, administration of *Jussiaea nervosa* extract exerted nephroprotective effects with mild to moderate congestion and peripheral preservation of kidney architecture (Figure 3).

IV. Discussion

This study has documented decreased hematological parameters and impaired renal function with plasma urea and creatinine elevated in Cd-exposed rats. These effects, which are dose dependent, were found to be ameliorated/restored by aqueous extract of *J. nervosa*. Significant decreases in hematological parameters (hemoglobin concentration and packed cell volume) observed in the present study is in corroboration with the findings of El-Demerdash *et al.* [16]. In a similar study, Horiguchi *et al* [17] reported decreased peripheral white blood cells in Cd administered rats. The toxicity and toxic effects of cadmium generally result from the binding of the metal with reactive and/or complex groups leading to inhibition of enzymatic process, possibly disturbing general growth, development and reproduction [3].

The significant decrease in the haemoglobin concentrations may be due to either an increase in the rate at which the hemoglobin is destroyed or to a decrease in the rate of haemoglobin synthesis. In addition to the established hemorrhage associated with Cd toxicity [18] decreased haematological parameters observed in the present study may be partly attributed to impaired renal functions. Impaired renal function in Cd-exposed rats, as evidenced by elevated plasma urea and creatnine as well as electrolyte imbalance suggests the likelihood of kidney damage as the cause of decreased haematological parameters.

It has been reported that kidney damage is associated with reduced erythropoietin production and excess breakdown of blood protein [19] with a resultant decrease in hematological parameters. However, it has been proposed that the mechanisms by which Cd induce anaemia include decreased iron absorption, distortion of erythropoiesis and hemolysis of red blood cells [20, 21]. Kidney damage has long been described to be the main problem for patients chronically exposed to cadmium [22]. This is in corroboration with the findings of the present study where Cd-exposed rats were observed to have elevated plasma urea and creatinine as well as electrolyte imbalance.

Cadmium induces kidney damage through the formation of cadmium-metallothionein (Cd-MT) complex. Cd-MT complex is filtrated in the glomerulus, and subsequently reabsorbed in the proximal tubulus where it remains and makes up the major part of the cadmium body burden. It has been proposed that the amount of cadmium in the kidney tubular cells increases during every person's life span. The severe congestion, infiltration of inflammatory cells, widespread injury on glomeruli, hemorrhage, pkynosis and excessive necrosis observed in the liver of Cd exposed rats (Figure 2) in comparison to the unexposed rats (Figure 1) and the preservation of kidney architecture with moderate congestion (Figure 3) observed after treatment with *Jussiaea nervosa* leave extract observed in the present study, suggests nephroprotective potentials of the extract. This reaffirms earlier suggestion of impaired kidney function as the cause of electrolyte imbalance and increased plasma urea and kidney. Although we did not encounter any study on the effect of *J. nervosa* in Cd-exposed rats, protective effects of some herbs have been reported. For instance, the hepatoprotective effect of onion and garlic extracts on cadmium (Cd)-induced oxidative damage in rats have been reported [23, 24].

Though the mechanism of the observed effects are not yet clear at this stage of the work, however, *Jussiaea nervosa*, like most of other plants contains phytochelatins which bind to metallic ions through metal chelation mechanism to form mercaptide complexes [25]. Cadmium is apparently bound reversibly to metallothionein (Cd-MT). Metallothionein has also been reported to play an important protective role in the heavy metal toxicity via the formation of metal–metallothionein complexes [26]. Thus it is possible that phytochelatin form complexes with Cd^{2+} and neutralize its toxicity [27].

V. Conclusion

The restoration of hematological parameters and renal function by aqueous extract of *J. nervosa* suggest its hematorpotective and nephroprotective potentials. We therefore conclude that regular consumption of *Jussiaea nervosa* leaf may be protective against Cd toxicity.

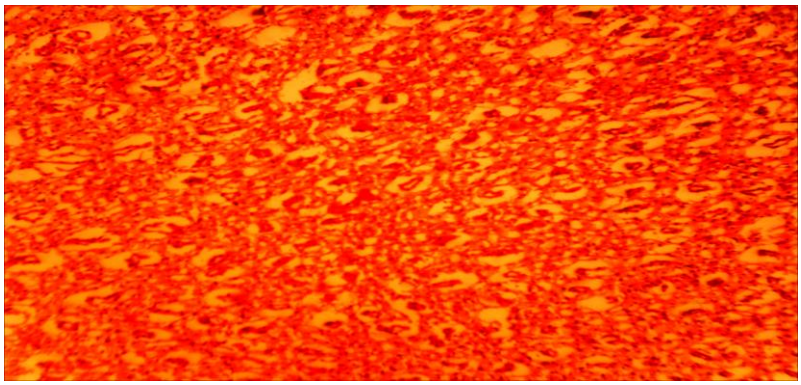

Figure1: Micrograph of the section of the kidney of albino rat which was fed with only water and rat feeds (NC). The section shows normal kidney architecture with well preserved glomeruli.

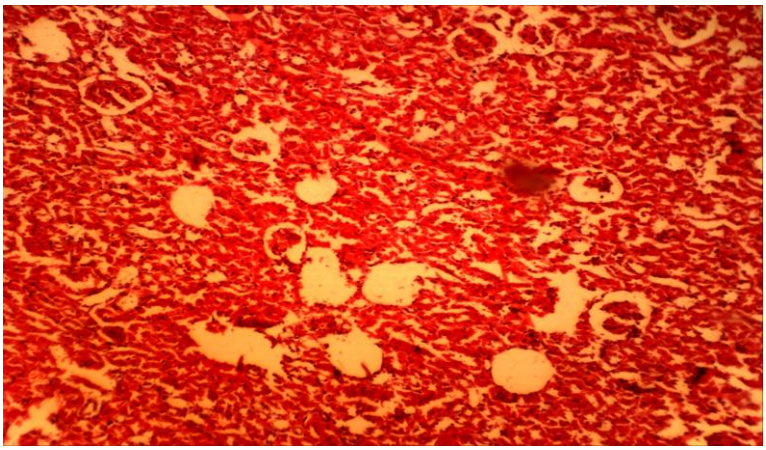

Figure 2: Micrograph of a section of the kidney of albino rat exposed to 12 mg/kg of Cd. The plate shows widespread injury on glomeruli and pkynosis , hemorrhage, severe congestion and extensive necrosis.

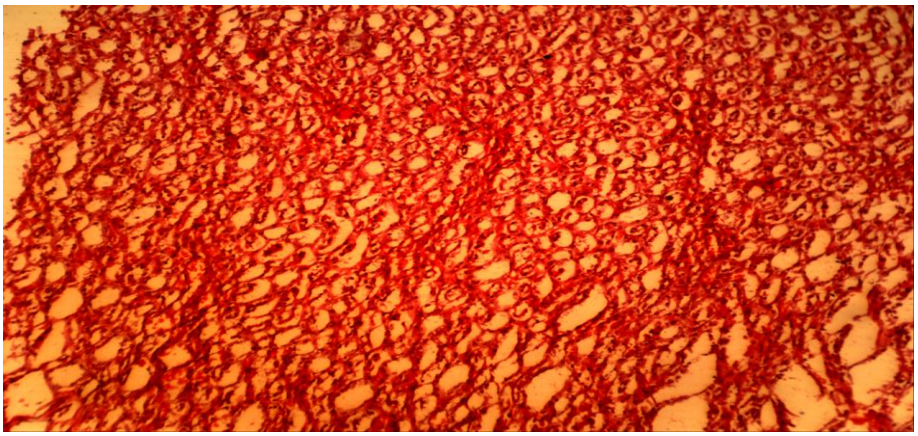

Figure 3: Micrograph of the section of the kidney of albino rat exposed to 12mg/Kg of Cd concentration and treated with Jussiaea nervosa water extract. The section shows preserved glomeruli.

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