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Research Paper

MHB-21 Ameliorates Cisplatin-induced Nephrotoxicity and improves Kidney Function in Rats.

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ABSTRACT
The high cost of standard medications, as well as their undesirable side effects, among other factors has necessitated the increasing search for alternative therapies for kidney disease, which have remained a huge burden on humanity. In the present study, the effect of a novel herbal formula- MHB-21 on cisplatin- induced nephrotoxicity was evaluated in albino rats as animal models. After acclimatization with laboratory conditions, twenty albino rats used for the study were randomly distributed into 5 groups (n=4). Rats in group 1 which served as normal control were only maintained on water. Rats in groups 2-5 (the test groups) were subjected to nephrotoxicity induction via a single intra-peritoneal administration of cisplatin at the dose of 5mg/kg body weight. Rats in group 2 were kept untreated (continued to receive only water like those in group 1), while rats in groups 3, 4 and 5 were treated with MHB-21 at respective oral doses of 400, 600 and 800mg/kg body weight. The treatments lasted for 14 consecutive days, at the end of which the animals were sacrificed. Blood samples were collected in plain sterile sample bottles, allowed to clot and centrifuged to obtain the serum used for measurement of urea, uric acid, creatinine and albumin. Kidney samples removed from the rats were fixed in 4% formaldehyde, subsequently processed and subjected to histological investigations. Results of the biochemical studies showed that the rats with cisplatin-induced toxicity, left untreated had the highest values for urea, uric acid and creatinine (17.47 ± 2.60mmol/L, 228.3 ± 1.72 µmol/L, and 172.3 ± 3.08 µmol/L respectively). Cisplatin toxicity-induced rats that were treated with MHB-21 showed dose-dependent values, each significantly lower ($P < 0.05$) than the values for the intoxicated but untreated rats. Cisplatin toxicity induced but untreated rats however, have albumin values that were significantly lower ($P < 0.05$) than those for the treated rats. Treatment with MHB-21 also improved the kidney histological features of the cisplatin intoxicated rats compared with the intoxicated but untreated ones, which showed marked distortions of the renal cells. It was thus concluded that MHB-21 has ameliorative effect on cisplatin-induced liver toxicity in rats and can thus be standardized for use as a kidney protective tonic.

KEY WORDS: MHB-21, Herbal formula, ameliorative effect cisplatin, Renal toxicity, rats.

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I. INTRODUCTION

Cisplatin [Cis-diaminedichloroplatinium (II)] is a drug commonly used in cancer chemotherapy. It has remained one of the most commonly used potent antineoplastic agents for the management of different types of cancers [17]. This drug, though an excellent anticancer agent has enjoyed limited clinical use as a result of its severe toxic effects as observed on the kidney and liver. It is easily absorbed and accumulated in the renal and hepatic cells [15] and [12], an observation which may account for its toxic impact on the organs.

Oxidative stress has been implicated as a key mechanism in cisplatin-induced tissue/organ toxicity [8]. Owing to this knowledge, antioxidants are at most times administered prior to cisplatin treatment to counter its toxicity [6]. Plants remain a great gift from nature to man. The use of these natural products that exhibit therapeutic properties is as old as human civilization. In recent years, there has been an increased interest in alternative therapies as a result of factors like high cost of standard medications as well as adverse side effects of such orthodox medicines. Another of such factors may also be the fact that medicinal plants and their products are usually cheaper and more accessible to many people in developing countries [14].

MHB-21 is a herbal product prepared by our group- a team of researchers from the School of Industrial and Applied Sciences at the Federal Polytechnic Nekede, Owerri, in Imo State of Nigeria, led by Dr. Josephat

Chukwudi Onwusonye. Preliminary phytochemical studies showed that the herbal product is rich in various compounds that show strong antioxidant and anti-inflammatory properties. In the present study, the effect of the product (whose constituents are summarized in table 1) on cisplatin-induced nephrotoxicity was evaluated in albino rats.

Table 1: Names of herbs used in the formulation of *MHB-21* and the parts Used

S/N	Botanical Name	Common Name	Parts Used
1.	*Carica Papaya*	Pawpaw	Seeds
2.	*Ocimum gratissimum*	Scent leaf	Leaves
3.	*Zingiber officinale*	Ginger	Rhizomes (roots)
4.	*Allium Sativa*	Garlic	Bulbs

II. MATERIALS AND METHODS

All the four plant materials were purchased from Nkwo Ukwu Ihiagwa Market in Owerri West L.G.A. of Imo State. The samples were identified by a Taxonomist in the department of Biological Sciences, Federal University of Technology, Owerri.

2.2 SAMPLE PREPARATION AND HERBAL FORMULATION

The mature *Carica papaya* fruit was cut open and the seeds were collected in a tray and allowed to dry under shed for two weeks, after which it was ground to a fine powder. Fresh leaves of *ocimum gratissium* were washed with clean water, air dried at room temperature for ten days and subsequently milled into fine powder. The roots of *Zingiber officinale* and the *Allium Sativa* bulbs were separately washed and dried under shed for two weeks after which they were separately milled into dry powder. Five hundred grams of each herbal powder was measured out separately and subsequently mixed together and homogenized in an electric blender to give 2 kg of mixed herbal powder.

2.3 SAMPLE EXTRACTION

The 2kg composed herbal powder was soaked in six litres of 95% methanol and allowed to stand on the bench with intermittent stirring. After 72 hours, the soaked material was filtered through 8µm Whatman filter paper. The resultant filtrate was concentrated to dryness in a rotary evaporator at 45^{oC}.

2.4 EXPERIMENTAL ANIMALS`

Breeding of the rats used in the study was done by our team. A total number of twenty male whistar albino rats was used in the study. The United States National Institute of Health Principles of Laboratory animals Care [9] were adhered to in the study.

2.5 EXPERIMENTAL DESIGN

The animals were distributed into five groups of four rats per group. The rats in group 1 did not receive cisplatin but rather kept on distilled water. Each rat in groups 2, 3, 4 and 5 was administered with a single dose of cisplatin (5mg/kg body weight) intraperitoneally at the beginning of experiment [7] and [11]. The cisplatin treated rats were left for 3 days for induction of kidney tissue damage [13]. On the 3^{rd} day, treatment started as follows: Rats in group 1 (normal control) were maintained on sterile water, rats in group 2 were also maintained on sterile water to serve as pathological control. Rats in groups 3, 4 and 5 were treated with 400, 600 and 800mg/kg of *MHB-21* respectively. All treatments were given once daily for 14 consecutive days. On the 15^{th} day, all animals were sacrificed and blood samples were taken for the measurement of biochemical parameters. Kidney samples were carefully removed and fixed in 4% formaldehyde for histological observation. Estimation of Urea was done by the modified Berthelot method [16]. Estimation of uric acid was done by the enzymatic colorimetric method [5]. Estimation of Creatinine was done by the colorimetric kinetic method [3]. Estimation of Serum albumin was done by the bromocresol green method [4]. Kidney histology was done according to the method described by Akparie [2].

2.6 STATISTICAL ANALYSIS

The data collected were subjected to Analysis of variance (ANOVA) implemented in SPSS statistics version 17.0. The means were separated using Duncan Multiple Range Test at the 0.05 level of significance.

III. RESULTS

The results of the biochemical evaluations are presented in tables 2-5, while those for histological studies are shown in Photomicrograph plates 1-5.

Table 2: Effects of *MHB-21* on Serum Urea Levels of Cis-platin Renal Toxicity-induced rats.

Groups	Treatment	Serum Urea (mmol/L)
1 control	(Distilled water)	6.15 ± 0.91^{a}
2	Cisplatin + Distilled water	17.47 ± 2.60^{b}
3	Cisplatin + MHB – 21 (400mg/kg b.w.)	8.16 ± 0.80^{a}
4	Cisplatin + MHB – 21 (600mg/kg b.w.)	7.58 ± 0.39^{a}
5	Cisplatin + MHB – 21 (800mg/kg b.w.)	6.95 ± 0.52^{a}

Values represent Mean ± SEM (n = 4)
a, b: Values with different superscript are significantly different (P < 0.05)

Table 3: Effect of *MHB-21* on Serum Uric Acid Levels of Cisplatin Renal Toxicity-induced rats

Groups	Treatment	Serum Uric acid (µmol/L)
1 control	(Distilled water)	146.5 ± 3.05^{a}
2	Cisplatin + Distilled water	228.3 ± 1.72^{b}
3	Cisplatin + MHB – 21 (400mg/kg b.w.)	165.8 ± 0.90^{c}
4	Cisplatin + MHB – 21 (600mg/kg b.w.)	152.3 ± 0.64^{a}
5	Cisplatin + MHB – 21 (800mg/kg b.w.)	132.0 ± 0.32^{a}

Values represent Mean ± SEM (n = 4)
a, b, c: values with different superscripts are significantly different (P < 0.05)

Table 4: Effect of *MHB-21* on Serum Creatinine Levels of Cisplatin renal toxicity induced rats

Groups	Treatment	Serum Creatinine (µmol/L)
1. control	Distilled water	86.5 ± 2.31^{a}
2.	Cisplatin + Distilled water	172.3 ± 3.08^{b}
3.	Cisplatin + MHB-21 (400mg/kg b.w.)	108.7 ± 1.96^{c}
4.	Cisplatin + MHB-21 (600mg/kg b.w.)	105.6 ± 0.93^{d}
5.	Cisplatin + MHB-21 (800mg/kg b.w.)	93.2 ± 0.86^{a}

Values represent Mean ± SEM (n = 4)
a - d: values with different superscripts are significantly different (P < 0.05)

Table 5: Effect of *MHB-21* on Serum albumin levels of Cisplatin renal toxicity-induced rats

Groups	Treatment	Serum albumin (g/L)
1. control	Distilled water	61.2 ± 2.05^{a}
2.	Cisplatin + Distilled water	29.8 ± 1.65^{b}
3.	Cisplatin + MHB-21	45.6 ± 0.83^{c}
4.	Cisplatin + MHB-21	50.6 ± 0.91^{d}
5.	Cisplatin + MHB-21	58.3 ± 0.23^{a}

Values represent Mean ± SEM (n = 4)
a - d: values with different superscripts are significantly different (P < 0.05)

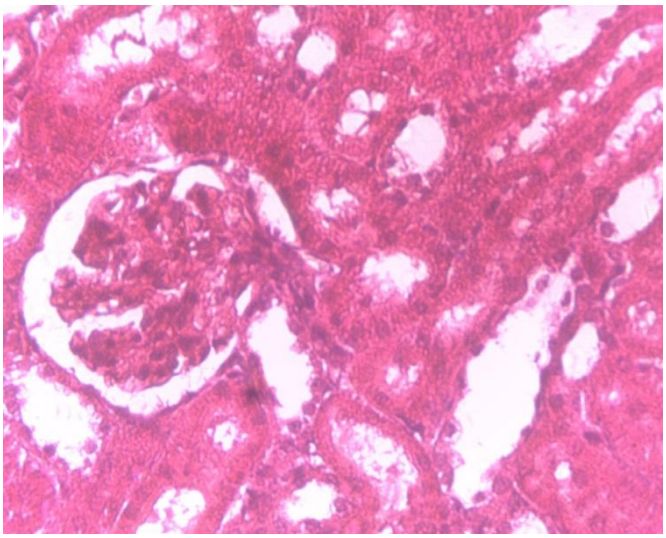

Plate 1: Photomicrograph of kidney of normal control rats showing well preserved glomerular and tubular cytoarchitecture (H&Ex400)

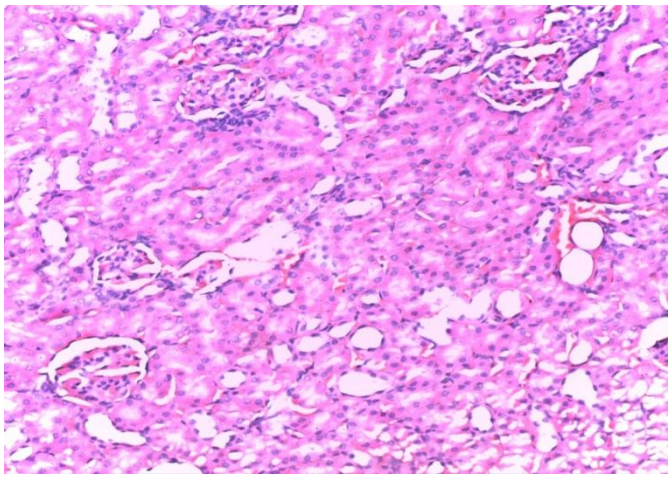

Plate 2: Photomicrograph of kidney of cisplatin- intoxicated rats kept on distilled water only, showing distorted glomerular and tubular cytoarchitecture (H&Ex400)

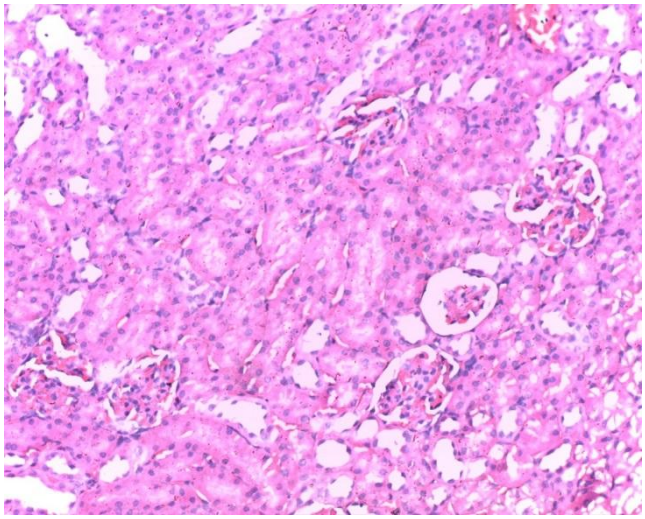

Plate 3: Photomicrograph of kidney of cisplatin -intoxicated rats treated with *MHB-21* (400mg/kg b.w.) showing fairly preserved glomerular and tubular cytoarchitecture (H&Ex400)

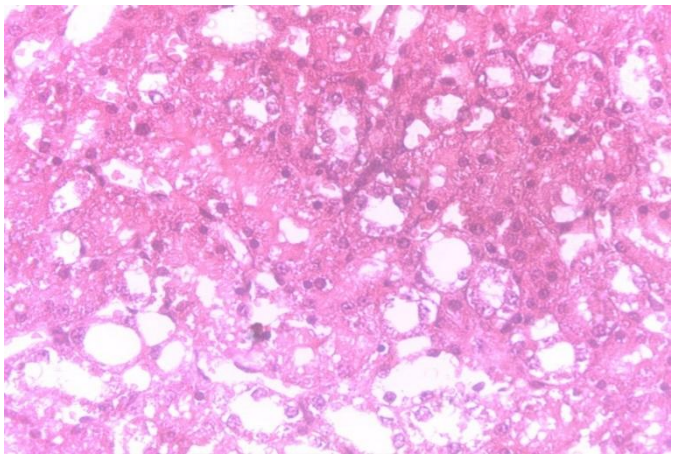

Plate 4: Photomicrograph of kidney of cisplatin-intoxicated rats treated with *MHB-21* (600mg/kg b.w.) showing well preserved glomerular and tubular cytoarchitecture (H&Ex400)

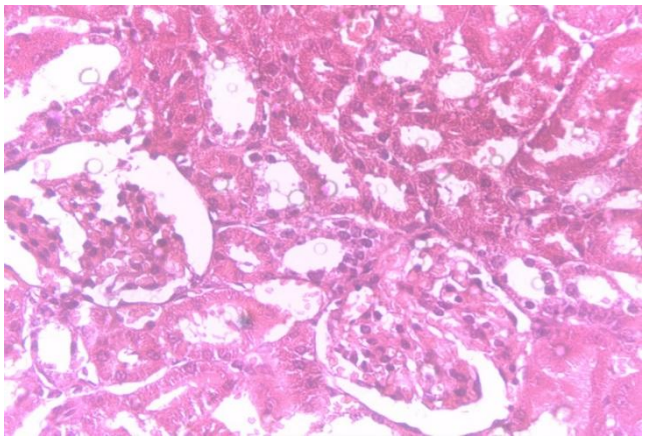

Plate 5: Photomicrograph of kidney of cisplatin-intoxicated rats treated with *MHB-21* (800mg/kg b.w.) showing well preserved glomerular and tubular cytoarchitecture (H&Ex400)

IV. DISCUSSION AND CONCLUSION

Plants have medicinal values owing to their phytochemical constituents, the most important among which are alkaloids, tannins flavonoids and phenols [1]. A number of factors ranging from life styles to environmental pollution contribute to increasing cases of human kidney diseases. In the present study, a single intraperitoneal dose of cisplatin (5mg/kg body weight) induced renal toxicity in the test rats after three days. This agrees with the report by Noori and Mahboob [10], that administration of ciaplatin to rats reduced the effectiveness of glomerular ultra-filtration. This in turn impacted negatively on the kidney function status of the rats. In this study, *MHB-21* has shown appreciable modulatory effects on the parameters of renal function under investigation. The herbal formula elicited significant dose-dependent reductions in serum urea levels of the cisplatin intoxicated rats (table 2). Its effects on serum uric acid and serum creatinine also followed the same pattern, with the cisplatin- intoxicated (but untreated) rats having values that are significantly higher ($P < 0.05$) when compared with rats in any other group (tables 3 and 4). Treatment with *MHB-21* however resulted to significant, dose-dependent elevations in the values of serum albumin. The marked reductions in serum urea, uric acid and creatinine and elevations in serum albumin levels are evidences of improvements in renal function as a result of treatment of the renal toxicity- induced rats with *MHB-21*. The results from the histological evaluations of the kidney tissues of the rats also lent more credence to the results obtained from biochemical studies. The rats in group 2, left untreated after induction of toxicity developed marked distortions in the cyto-architectural features of the kidney. Treatment with *MHB-21* however, resulted to marked improvements in these cyto-architectural features of the kidneys of the test rats. From these observations it is hereby concluded that the herbal formula has remarkable protective effect against cisplatin-induced kidney toxicity in rats and could be standardized for the management of other human kidney diseases. This observed nephroprotective efficacy exhibited by *MHB-21* may be attributed to among other factors, a high content of antioxidants and microelements that offer protections against cellular toxicity.

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