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THE EFFECT OF *HYPHEANE THEBAICA* EXTRACT ON GENTAMICIN INDUCED RENAL FAILURE IN RATS

Ebtehal El Taher[1*], Sania Shaddad[2], A. K. Muddathir[3], Nuha M.E. Agabna[2], Amna E. H. Mohammed[4] and Afaf I Abualgassim[5]

[1]Omdurman Maternity Hospital.

[2]Dept. of Medicine (Pharmacology), Faculty of Medicine, University of Khartoum. P.O. Box 102.

[3]Dept. of Pharmacognosy, Faculty of Pharmacy, University of Khartoum. P.O. Box 1996.

[4]Dept. of Pharmacology & Toxicology..Medicinal and Aromatic Plants and Traditional Medicine Research Institute. National Center for Research.

[5]Dept. of Pathology Faculty of Veterinary Medicine University of Khartoum.

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***Corresponding Author**
Ebtehal El Taher
Omdurman Maternity Hospital.

ABSTRACT

The effects of hot water infusion of the dried endocarp of Hypaene thebaica were examined on a group of albino rats. Animals were divided into four groups of six each; one was control, the second received gentamicin 100mg/kg to induce renal damage and served as positive control. The third group received gentamicin 100mg/ kg and 0.57mg/kg H. thebaica oral concurrently for eight days. The fourth group was given the extract oral for 21 days. Results showed that urea creatinine and potassium levels were significantly increased in group two and three indicating that H. thebaica did not protect the renal damage. However histopathologal study detected mild amelioration of nephronal damage. Liver enzymes were slightly elevated in the treated groups. Haematological parameters were restored in the H. thebaica group denoting that the extract had haematinic effects.

KEYWORDS: Hypheane thebaica, gentamycin, renal failure, rats.

INTRODUCTION

Hyphaene thebiaca (L) Mart family arecaceae (palmae) is commonly known as Doum palm. The fruits are used in folk medicine for the treatment of spleen, stomach, abdominal pain and wounds, also other parts of the plant are used in conjunctivitis (EL-Gazali, 1998). It is

documented that extracts are used locally in the treatment of bilharzias, heamaturia, bleeding especially after childbirth and also as hematinic agent (Adaya, 1977; Von-Maydell, 1986).
The plant contains low levels of tannins, steroids, and moderate amounts of flavnoids, sapponins, carbohydrates, cardiac glycosides, terpines and terpinoids besides various metals (Aboshora 2014 Auwal, 2013, EL-Gazali,; 1998).

Pharmacological studies of Doum seeds extracts on the isolated smooth muscles showed that chloroform extract of the seeds inhibits the spontaneously contracting rabbit jejunum by 40% (Eltayeb 2009). High Anti-inflammatory activity was found in the chloroform and ethanolic extracts of the seeds, roots and leaves (Eltayeb 2009). Similar results were obtained by Elsiddig 1996. *H. thebaica* at 500 mg/kg showed significant amelioration of experimentally induced irritable bowel syndrome IBD, which may be attributed to its antioxidant and anti-inflammatory properties (Shalaby, 2013).

The crude mesocarp extract was found to produce hypoglycaemia after three weeks in normo-gylcaemic rats (Auwal, 2013). Abdel-Rahim 2011 observed that the ingestion of methanolic extract significantly reduced the high level of blood glucose in the diabetic rats. Administration of the water fraction improved glucose and insulin tolerance and significantly lowered blood glycosylated hemoglobin levels, however both urea and creatinine levels in serum were highly significant (Salib et al 2013)

The plant extract was also found to reduce total cholesterol and non HDL lipoproteins in normocholestremic rats (Hetta, 2006). Modu. (2000/2001) using aqueous pulp extract of *hyphaene thebaica* (L) Mart found the extract to be hypolipidemic but nontoxic to both liver and kidney. Kamis, (2000) reported that at high concentration, the extract was hypolipidemic, hepatotoxic and nephrotoxic. Daily oral administration lasted for three weeks results revealed a significant ($p<0.05$) decrease in levels of triglycerides, cholesterol and total lipids in addition to total proteins and albumin compared to the control. Also the levels of potassium ions were significantly ($p<0.05$) decreased.

Regarding antimicrobial activity, the aqueous extract of the dormant fruits was tested for in vitro antifungal activity using the agar dilution method. The extract was found to produce significant antifungal activity against a wide range of fungal isolates including Candida albicans, Microsporum gypseum, Trichophyton rubrum, Mucor sp., Fusarium solani and Aspergillus niger (Irobi & Adedayo, 1999). Dosumu (2006) and Mohamed (2010) found that

extract and its fractions had significant action against pathogenic bacteria, *Staphylococcus aureus*, *Eschericia coli*, *Bacillus subtilis*, *Pseudomonas aeruginosa* and *Klebsiella pneumonia*.

These effects were attributed in-part to antioxidant capacity, however, the phenolic content was not high in the extracts but it had significant antioxidant activity (Mohamed et al 2010 Hsu, 2006)

Objectives

- To investigate the H. thebaica extract on renal, liver and haematological parameters against gentamicin-induced nephrotoxicity in rats.
- To study histopathological effects of the plant's extracted.

Methodology

1. Materials

1.1. *Animals*

Male and female albino rats, weighing 105 -220 gm were obtained from National Center for Research, Khartoum, Sudan and were kept at animal house of Faculty of Pharmacy, University of Khartoum. All animals were kept at room temperature (26±1°C) and freely accessed to food and water.

1.2. *Drugs*

Gentamicin, diethyl ether, formaldehyde.

1.3. *Equipments*

Sysmex KX-21, Hitach analyzer.

2. Methods

2.1. *Preparation of plant materials*

The dried fruit (pulp) was obtained from Bahry Market (clean and newly harvested fruits were selected). The endocarp were finely powdered and kept for further uses.

2.2. *Preparation of extracts*

Twenty grams (20g) of the powdered endocarp were infused in 100 ml hot distilled water, left for 10 minutes, then the solution was filtered, left to cool (room temperature). The solution was freshly used.

2.*3. Assessment of nephroprotective activity against gentamicin-induced nephrotoxicity*

Nephrotoxicity was induced by gentamicin (100mg/kg). Gentamicin was injected by intramuscular route concomitantly with plants extracts administered orally. Blood samples were drawn from orbital plexuses under light diethyl ether anesthesia into heparinized capillary tubes. Serum was separated by centrifugation for 15 minutes at 3000 rpm .The serum samples were kept frozen at -20°C until used for measurement of renal and liver function parameters.

2.4. Hematological methods

For hematological measurement, blood samples were collected from rats in dry clean bottles containing ethelene diamine tetra acetic acid (EDTA) as described by Schalm (1965).

Haemoglobin concentration (Hb), packed cell volume (PCV) , red blood cell count (RBC), mean corpuscular volume (MCV), mean corpuscular heamoglobin concentration (MCHC) were determined by using Sysmex KX-21.

2.5. Experimental design

The animals were divided into four groups of six animals, group1 was control, a group2 was given gentamicin I.M 100mg/kg, and group3 was given gentamicin I/M concurrently with extract oral. The group4 was given the extract orally (0.57 mg/kg**)** for 21 days.

2.6. Statistical analysis

The observation in each group were compiled and tabulated for the assessment of mean and standard error of mean (Mean ± SEM). Statistical comparison between different groups was done using one-way analysis of variance (ANOVA) and T-test using SPSS program version 17.0).

RESULTS

Table 1: Renal parameters.

Group		Creatinine mg/dl	Urea mg/dl	Na Mmol	K Mmol
Control	Day 0	0.39±0.01	51.33 ±1.17	141.0±0.37	4.73±0.2
	Day 8	0.40±0.0	49.67 ± 1.05	141.67±0.21	4.43±0.18

Gentamicin IM 100mg/Kg	Day 0	0.33±0.05	67.17 ±3.63	137.33±3.29	4.57±0.19
	Day 8	0.63±0.02*	79.00±1.32*	142.33±084	3.77±0.27*
Gentamicin IM + Extract	Day 0	0.37±0.02	72.03± 3.35	142.67±0.21	3.67±0.02
	Day 8	0.7±0.1*	99.33±8.71*	142.23±1.3	4.07±0.04*

*significant p<0.05 N= 6

Table 2: liver parameters.

Group		AST IU/ml	ALT IU/ml	Albumin g/dl
Control	Day 0	93.87±5.12	53.39±4.09	3.3±0.0
	Day 8	90.9±4.35	56.23±2.38	3.63±0.17
Gentamicin IM 100mg/Kg	Day 0	73.0±2.55	41.51±4.45	3.7±0.2
	Day 8	145.37±4.83	48.53±7.18	3.27±0.06
Gentamicin IM + Extract	Day 0	77.1±3.19	49.63±4.72	4.17±0.11
	Day 8	111.97±1.62*	49.18±1.43	3.3±0.2*

*significant p<0.05 N= 6

Table 3: Haematologial parameters.

Group		Hb g/dl	RBC X106µ/L	MCHC g/dl	MCV FL	PCV %
Control	Day 0	13.77±0.25	7.47±0.5	31.63±0.33	58.47±0.52	43.6±1.06
	Day 8	14.3±0.11	7.3±0.01	32.23±0.16	58.73±0.09	44.33±0.13
Gentamicin IM 100mg/Kg	Day 0	13.3±0.5	7.83±0.22	29.43±1.6	58.03±0.55	45.4±1.11
	Day 8	11.77±0.33*	6.8±0.39*	30.17±1.35	58.1±031	39.58±2.36*
Gentamicin IM + Extract	Day 0	12.97±0.94	7.7±0.23	32.23±0.2	56.97±0.49	43.83±0.93
	Day 8	12.27±0.94	6.71±0.55	30.3±0.32	60.93±0.46*	40.97±3.57

Table 4: results after 21 days oral administration of the extract.

Parameter	Day 0	Day 21
Creatinine mg/dl	0.28±0.03	0.3±0.03
Urea mg/dl	48.84±1.83	48.84±1.83
AST IU/ml	145.33±6.5	166.73±9.07
ALT IU/ml	69.43±9.14	69.43±9.14
Albumin g/dl	3.63±0.06	3.9±0.11
Haemoglobin g/dl	11.82±0.72	12.5±0.57
RBC $x10^6$µ/L	6.85±0.43	6.92±0.2
MCHC g/dl	29.18±0.47	30.8±0.57
MCV (FL)	5902±0.43	58.54±0.8
PCV (%)	40.58±2.66	40.54±1.48

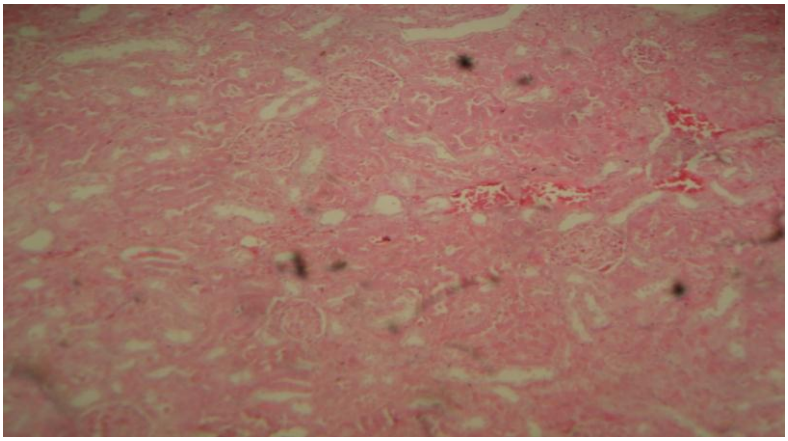

Figure 1: Kidney from rats that received Gentamicin (100 mg/kg) for 8 days.

Displays evidence of nephrotoxicity, there is tubular destruction seen as coagulative necrosis and haemorrhage. The glomeruli are less cellular with hyaline cast.

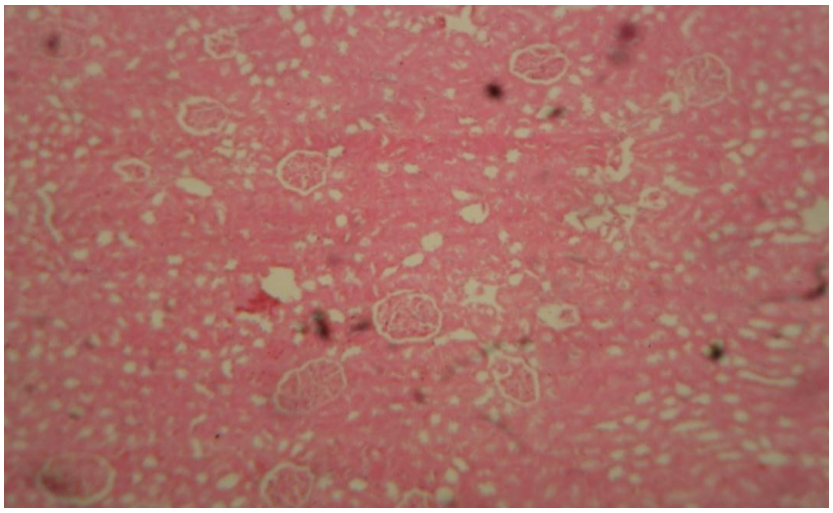

Figure 2: Kidney from rats received *Hyphaene thebacia* (0.57 mg/kg) for 21 days

Shows little tubular destruction but the glomeruli are not affected

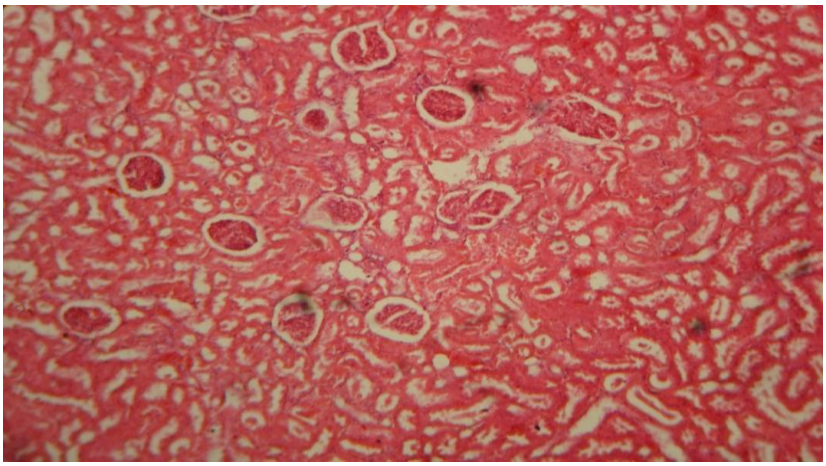

Figure 3: Kidney from rats received *Hyphaene thebacia* (0.57 mg/kg) for 8 days concomitantly with Gentamicin (100 mg/kg)

There is tubular destruction and hyaline cast.

DISCUSSION

The effect of H. thebaica on renal system have been investigated in many studies, some found the plant to be nephrotoxic. This study examines the effect of the plant on gentamicin induced renal failure. This study found that H. thebaica extract given orally at a dose of (0.57 mg/kg) failed to protect the kidney from damage by gentamicin (table 1) Creatinine and urea levels significantly raised in the gentamicin groupsand the group protected by the extract. Potassium levels were significantly elevated in the a fore mentioned groups indicating that extract gave no protection to acute renal failure. However, the group that received the extract alone for 21 days displayed no change in urea, creatinine, sodium and potassium levels (table 4). Close results were observed by Zanna et al (2008) in regard to urea and potassium levels, but noticed increased creatinine levels. Kamis (2003) administering higher doses of the extract (0.5, 1.0 & 2.5mg/kg) reported elevated urea and decreased potassium levels.

A study conducted by AbdeEl-moniem et al 20015 found that H. thebaica protected rats from renal damage in strptozotocin induced diabetes mellitus rats besides reducing glucose levels. This effect was significant when animals were administered the extract prior to induction of diabetes compared to animals given the extract after development of diabetes. Tohamy et al. 2016 and Shehu et al., 2014 documented that H. thebaica protected diabetic rats from renal and hepatic deterioration.

Table 2 shows the effect of the extract on liver enzymes, AST was significantly raised in the group that received gentamicin and H. thebaica. ALT levels showed no changes in all three groups, but albumin was reduced in the group that received gentamicin and the extract. Similar results were observed by Zanna (2008).

The extract had significant haematinic properties, and seemed to protect against changes in blood parameters induced by renal failure. Haemoglobin concentration, red blood cell count, mean corpuscular volume and packed cell volume were all close to normal in the group that received H. thebaica compared to reduced parameters in the gentamicin group (table 3). Similar results were observed by other researchers. Moreover, Auwal 2013 stated that white blood cells count increased with administration of the extract, specifically, lymphocytes, monocytes, eosinophiles and basophiles were increased.

The histopathological study reflected that H. thebaica mildly ameliorated the renal damage caused by gentamicin. The glomeruli were not affected in the group that received the extract.

Shalby et al 2012 also noted that the extract protected the morphology of the kidneys in cyclosporine induced renal failure.

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