

## Hepatoprotective effect of Aqueous Extract of *Hyphaene thebaica* (Doom Fruit) on Paracetamol-Induced Liver Injury in Albino Rats

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**Abstract:** Level of liver marker enzymes AST, ALT and ALP and level some biochemical parameters Albumin, Urea and Creatinine were assayed in rats fed with aqueous extract of *Hyphaene thebaica*. This study was done to determine the hepatoprotective activity of aqueous extract of *Hyphaene thebaica* in paracetamol-induced liver injury albino rats. Acute toxicity study (LD50) was first evaluated in rats before the commencement of the main study. Twenty five albino rats weighing between 90-120g were used. They were divided into five (5) groups of five (5) rats each. Rats in group one served as the normal control, group two as positive group they were treated with standard drug Silymarin at a dose of 50mg/kg, groups 3 and 4 were administered the aqueous extract daily by intubation for seven days at 250mg/kg for group and 500mg/kg for group four respectively. All treatments were carried out for 7 days and PCM intoxication was done 3 hours after the last dose. All animals were euthanized twenty four (24) hours after the last treatment; blood was collected, and used for estimation of biochemical parameters such as liver enzymes (alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP), and level of serum albumin, Creatinine and urea. In the experiment carried out there was no significant difference ( $P>0.05$ ) in terms of ALP of all the groups in comparison to the control group. Aqueous extract of *H. thebaica* however showed significant increase at  $P<0.05$  in this protein, both urea and creatinine levels were increased significantly by PCM but were both decreased significantly by the administration of aqueous extract of *H. thebaica*.

**Keywords:** *Hyphaene thebaica*; Hepatotoxicity; Hepatoprotection; Acetaminophen.

## INTRODUCTION

The liver plays a key role in transforming and detoxifying chemicals and this makes it susceptible to damage. Similarly certain medicinal agents, when taken in overdoses and sometimes even when introduced within therapeutic ranges, may injure the organ. Chemical agents, such as laboratories and industries chemicals, natural chemicals and herbal remedies can induce hepatotoxicity [1]. Chemicals that cause liver injury are called hepatotoxins. Hepatotoxicity (from hepatic toxicity) implies chemical-driven liver damage. Drug-induced liver injury is a cause of both acute and chronic liver disease.

Liver diseases which are still a global health problem may be classified as acute or chronic hepatitis (inflammatory liver diseases), hepatitis (non inflammatory diseases) and cirrhosis (degenerative dis+order resulting in liver fibrosis). Total deaths

worldwide from liver Cirrhosis, liver cancer and other liver disease rose by 50 million per year over 2 decades, according to the first-ever World Health Organization study of liver disease mortality. Chronic hepatic diseases stand as one of the foremost health trouble worldwide, with liver cirrhosis and drug-induced liver injury accounting for with leading cause of death in western and developing countries [1]. Unfortunately, treatments of choice for liver diseases are controversial because conventional or synthetic drugs for the treatment of these diseases are insufficient and sometimes cause serious side effects [1].

Since ancient times, mankind has made use of plants in the treatment of various ailments because their toxicity factors appear to have lower side effects [3]. Many of the currently available drugs were derived either directly or indirectly from medicinal plants. Recent interest in natural therapies and alternative

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medicines has made researchers focus on traditional herbal medicine. In the past decade, attention has been centered on scientific evaluation of traditional drugs of plant origin for the treatment of various diseases. Due to their effectiveness, with presumably minimal side effects in terms of treatment as well as relatively low costs, herbal drugs are widely prescribed, even when their biologically active constituents are not fully identified [4].

Hundreds of plants have been so far examined to be taken for a wide spectrum of liver diseases [5]. Natural products, including herbal extracts, could significantly contribute to recovery processes of the intoxicated liver. Plants such as *Silybum marianum*, *Glycyrrhiza glabra*, *Phyllanthus species (amarus, niruri, emblica)*, and *Picrorhiza kurroa* have been widely and most of the time fruitfully applied for the treatment of liver disorders, exerting their effects via antioxidant-related properties [6].

*Hyphaene thebaica* belongs to the family Arecaceae commonly known as doum palm, dum nut or gingerbread tree. Important economic species also occur in India and other parts of Asia and Africa [7]. Roots, fruits and seeds of doum palm are used for the management of jaundice, intestinal colic, hematuria, inguinal hernia bilharzias, and hypertension and sore eyes in livestock. The mesocarp is credited with diuretic properties [8] and also has been found to possess antioxidant property [9].

This study was done to determine the hepatoprotective effect of aqueous extract of *H. thebaica* in paracetamol-induced liver injury in albino rats.

## MATERIALS AND METHOD

### Plant collection and identification

Fresh fruit of the *Hyphaene thebaica* was purchased from Gamboru market in Maiduguri Borno state, Nigeria. The fruit were authenticated by a taxonomist at the Department of Biological Sciences, University of Maiduguri. Voucher specimen of this plant was kept in the toxicology laboratory, University of Maiduguri for reference.

### Preparation of Sample

Fresh fruit of the *H. thebaica* were collected and ground to fine powder and stored in a plastic container. 100g of the powder dissolved in 500ml of distilled water and left for 24 hours on a plane surface with continues shaking at intervals disturbance at room temperature 37°C, it was sieved after 24 hours using muslin cloth and separated from debris after which it was evaporated using a rotary evaporator. 24g of the aqueous extract was obtained after extraction. The sample (extract) was stored in the refrigerator at 40°C.

### Experimental Animals

34 albino rats of both sexes weighing between 90-120g were used, Animals were obtained from the Animal house, Department of Biochemistry, University of Maiduguri, Nigeria. The animals were housed in well-ventilated Cages and fed with commercial laboratory diet and water ad libitum.

### Acute Toxicity study

This was carried out according to the method of described by Lorke, 1983. Where 9 albino rats were used for the phase 1, the rats were divided into three groups of each three rats each. Groups 1-3 were treated with the aqueous extract of *H. thebaica* at different doses of 10mg/kg, 100mg/kg, and 1000mg/kg respectively. The rats were observed for signs of toxicity and mortality for the first 4hours and for 24hours.

In the phase II, 3 rats were used; the rats were divided into three groups of 1 rat each. Groups were treated with the aqueous extract of *H. thebaica* at different doses of 1600mg/kg, 2900mg/kg and 5000mg/kg respectively. The rats were observed for signs of toxicity and mortality for the first 4hours and for 24hours.

### Hepatoprotective activity study

Animals were divided into five groups of five rats each. Rats in group 1 served as normal control and administered distilled water, group 2 were administered distilled, group 3 served as positive control and they treated with sylimarin drug at a dose of 50mg/kg, and group 4 and 5 were administered with the aqueous extract *H. thebaica* daily by intubation for seven days with single dose of 250mg/kg and 500mg/kg respectively. All treatments were carried out for 7 days and paracetamol (PCM) intoxication with 2g/kg of pcm was done on the seventh, day 3 hours after the last administration to all groups except group 1. All animals were then euthanized by cervical dislocation twenty four (24) hours after pcm induction. Blood sample was collected in plain container, and allowed to clot before centrifuged at 4000rpm for 20 minute and serum harvested. Serum harvested was used for estimation of biochemical parameters such as liver enzymes (alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP), and level of serum albumin, Creatinine and urea.

### Determination of Biochemical Parameters

AST and ALT were assayed using kits based on the method of Reitman and Frankel [22]. ALP was assayed using kits based the method of Klein *et al.* [23]. Albumin was assayed by the bromocresol green method of Doumas *et al.* [17]. Serum Creatinine was assayed by Jaffe method [24] and serum Urea was assayed by Kjeldahl [25].

## RESULT AND DISCUSSION

The results from acute toxicity study in phase I and phase II indicate that the LD<sub>50</sub> of aqueous extract of Doum fruit (*H. Thebaica*) is >5000mg/kg. There were no sign toxicity and mortality after 24 hours.

The level of both AST and ALT was significantly increased by PCM but has also been seen to be decreased significantly by the extract, the decrease in the level of both AST and ALT by the extract this may due to the presence of polyphenolic compound as reported by Sharaf *et al.* [10], the increase in the level of AST and ALT by the PCM is because PCM is regarded as a safe drug at the therapeutic dose but at higher doses, PCM can cause centrilobular necrosis that eventually leads to liver failure [11]. The major advantage of PCM model is that it is a clinically relevant model and is a dose dependent hepatotoxicant [12]. The major portion of PCM dose is conjugated with glucuronic acid or sulfate and the rest is converted into reactive metabolite N-acetyl-p-benzoquinoneimine (NAPQI) through cytochrome P450 enzymes [13]. At therapeutic dose, NAPQI is conjugated with reduced glutathione to form mercapturic acid which is excreted in urine [14]. However, in case of overdose, excess NAPQI depletes GSH content and binds covalently to hepatic cellular proteins resulting in mitochondrial dysfunction and mitochondrial oxidative stress that eventually induces necrosis and apoptosis of hepatocytes [15, 16].

ALP level is increased in conditions like: rapid bone growth, bone disease, vitamin D deficiency and liver damage. There was no significant difference (P>0.05) in terms of ALP of all the groups in comparison to the control group this is similar to the report of Smialovics [17] where there were no significant in ALP level in CCL4 induced hepatotoxicity in rats. This may due to the fact that ALP is associated with cholestasis and since PCM metabolism does not involve cholestasis, this parameter was not affected.

Cell damage in the absence of cholestasis cause relatively little release of hepatic alkaline

phosphatase, presumably because the enzyme is firmly bound to the membrane of the liver cell and therefore does not leak from damage cells into the blood as readily as soluble cytoplasmic enzyme [18].

Serum albumin was significantly decreased by PCM and this indicates liver damage since albumin is mainly produced by the liver. Aqueous extract of *H. thebaica* however showed significant increase at P<0.05 in this protein which can be compared with that of the standard group.

Nasir *et al.* [19] reported that there were significant decrease in serum total protein and albumin after administration of *A. Pariculeta* aqueous leaf extract in CCL4 induced-hepatotoxicity in rats.

Urea and creatinine are parameters used to assess kidney function. In this study, both urea and creatinine levels were increased significantly by PCM but were both decreased significantly by the administration of aqueous extract of *H. thebaica*.

In addition, the extract had no harmful effects on liver and kidney function tests as it improves serum levels of AST, ALT, and ALP. Serum albumin was significantly decreased by PCM and this indicates liver damage since albumin is mainly produced by the liver. Aqueous extract of *H. thebaica* however showed significant increase at P<0.05 in this protein which can be compared with that of the standard group.

The increase in serum protein and globulin might be due to the improvement of liver functions. Creatinine measurements are most useful in evaluating renal function. Unlike urea, creatinine is not affected by hepatic function. In a study using ethanolic extract of the plant [21], found that at high concentration, the plant is hypolipidemic, hepatotoxic and nephrotoxic. However [21], reported that aqueous pulp extract of *hyphaene thebaica* was hypolipidemic but nontoxic to both liver and kidney. This difference in response might be due to the differences in type of the doum extract or their dosages.

**Table-1: Acute toxicity study (LD<sub>50</sub>) of aqueous extract of Doum fruit in phase I and phase II (Lorke's method)**

Phases	Group	Rats	Dose	Observation
Phase I	A	1	10mg/kg	-
		2	10mg/kg	-
		3	10mg/kg	-
	B	1	100mg/kg	-
		2	100mg/kg	-
		3	100mg/kg	-
	C	1	1000mg/kg	-
		2	1000mg/kg	-
		3	1000mg/kg	-
Phase II	A	1	1600mg/kg	-
	B	1	2900mg/kg	-
	C	1	5000mg/kg	-

LD<sub>50</sub> > 5000mg/kg  
- Signifies no death

**Table-2: Hepatoprotective activity study of aqueous extract of Doum fruit on paracetamol-induced liver injury albino rats**

Group	Dose	AST (U/l)	ALT (U/l)	ALP (U/l)	ALBUMIN (mmol/L)	UREA (g/L)	CREATININE (umol/L)
CONTROL	1ml/kg	0.11± 0.01	0.13± 0.00	21.94± 0.32	118.62±19.00	0.27±0.03	0.33± 0.007
PCM	2g/kg	0.66± 0.25 <sup>a</sup>	0.16± 0.01	18.77± 2.36	95.88± 10.39 <sup>a</sup>	0.46± 0.02 <sup>a</sup>	0.46± 0.02 <sup>a</sup>
SYLM	50mg/kg	0.18± 0.01 <sup>b</sup>	0.15± 0.03	16.98± 3.06	134.07±23.53 <sup>b</sup>	0.55± 0.05 <sup>a</sup>	0.57± 0.12 <sup>a</sup>
AQU 1	250mg/kg	0.13±0.02 <sup>b</sup>	0.16±0.01	23.29±8.13	139±25.33 <sup>b</sup>	0.39±0.04 <sup>b</sup>	0.34±0.05 <sup>b</sup>
AQU 2	500mg/kg	0.11±0.05 <sup>b</sup>	0.15±0.01	16.71.00±1.55	86.09±0.66 <sup>a</sup>	0.25±0.05 <sup>b</sup>	0.28±0.03 <sup>b</sup>

Values expressed as mean ± SEMs control at  $p < 0.05$ , b – statistically significant to PCM at  $P < 0.05$ .

## CONCLUSION

From the acute toxicity study, the LD<sub>50</sub> of the extract was estimated to be greater than >5000mg/kg. The aqueous extract of *H. thebaica* at different doses of 250mg/kg and 500mg/kg is relatively safe following acute oral administration and may have hepatoprotective activity against paracetamol-induced liver injury in Albino rats.

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