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Hepatoprotective effect of aqueous extract of *Hyphaene thebaica (*Doum fruit*)* on paracetamol-induced liver injury in albino rats

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Abstract

This study was done to determine the hepatoprotective activity of aqueous extract of *Hyphaene thebaica* in paracetamol-induced liver injury in albino rats. Level of liver marker enzymes such as AST, ALT and ALP and level of some biochemical parameters such as albumin, urea and creatinine were assayed in rats fed with aqueous extract of *Hyphaene thebaica*. Acute toxicity study (LD_{50}) was first evaluated in rats before the commencement of the main study. Silymarin at a dose of 50 mg/kg was used as positive control. The aqueous extract of *Hyphaene thebaica* was administered to animals daily by intubation for seven days at 250 mg/kg and 500 mg/kg. All treatments were carried out for 7 days and paracetamol intoxication was done 3 hr after the last dose. All animals were euthanized 24 hr after the last treatment, blood was collected and used for estimation of biochemical parameters such as alanine amino transferase (ALT), aspartate amino transferase (AST), and alkaline phosphatase (ALP), and level of serum albumin, creatinine and urea. The results indicated that there was no significant difference ($p > 0.05$) in levels of ALP in all the groups in comparison to the control group. Both urea and creatinine levels were increased significantly by paracetamol intoxication but both were decreased significantly by the administration of aqueous extract of *H. thebaica.*

Keywords: *Hyphaene thebaica*, hepatotoxicity, hepatoprotection, acetaminophen

1. 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 laboratory and industrial 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 disoorder 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 [2].

Since ancient times, humankind 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 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 used, even when their biologically active constituents are not fully identified [4].

Hundreds of plants have been examined so far, 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

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credited with diuretic properties [8] and also have 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.

2. Materials and methods

2.1. Plant collection and identification

Fresh fruit of the *Hyphaene thebaica* was purchased from Gamboru market in Maiduguri Borno state, Nigeria. The fruits 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.

2.2. Preparation of Sample

Fresh fruit of the *H. thebaica* were collected and ground to fine powder and stored in a plastic container. 100 g of the powder was mixed in 500 mL of distilled water and left for 24 hr with continuous shaking at fixed intervals at room temperature 37 °C. The mixture was filtered after 24 hr using muslin cloth and separated from debris after which it was evaporated using a rotary evaporator. 24 g of the aqueous extract was obtained after extraction. The sample (extract) was stored in the refrigerator at 4 °C until further use.

2.3. Experimental animals

Thirty four albino rats of both sexes weighing between 90 – 120 g 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.*

2.4. Acute toxicity study

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

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

2.5. 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 water, group 3 served as positive control and they were treated with silimarin drug at a dose of 50 mg/kg, and group 4 and 5 were administered with the aqueous extract of *H. thebaica* daily by intubation for seven days with single dose of 250 mg/kg and 500 mg/kg respectively. All treatments were carried out for 7 days and paracetamol (PCM) intoxication with 2 g/kg 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 24 hr after PCM induction. Blood sample was collected in plain container, and allowed to clot before centrifuged at 4000 rpm for 20 min and serum was 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.

2.6. Determination of biochemical parameters

AST and ALT were assayed using kits based on the method of Reitman and Frankel (1957) [10]. ALP was assayed using kits based on the method of Klein et al., (1960) [11]. Albumin was assayed by the bromocresol green method of Doumas et al., (1971) [12]. Serum creatinine was assayed by Jaffe method (1886) [13] and serum urea was assayed by Kjeldahl (1883) method [14].

2.7. Statistical analysis

The data are expressed as mean ± SEM. One-way analysis of variance was used to analyse to differences amongst the groups. $p < 0.05$ was considered as the significant difference.

3. Result and discussion

The results from acute toxicity study in phase I and phase II indicated that the LD_{50} of aqueous extract of Doum fruit (*H. Thebaica*) is more than 5000 mg/kg. There were no sign of toxicity and mortality after 24 hr.

The level of both AST and ALT was significantly increased by PCM but decreased significantly by the extract. The decrease in the level of both AST and ALT by the extract may be due to the presence of polyphenolic compounds as reported by Sharaf et al. [15]. 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 [16]. The major advantage of PCM model is that it is a clinically relevant model and is a dose-dependent hepatotoxicant [17]. 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 [18]. At therapeutic dose, NAPQI is conjugated with reduced glutathione to form mercapturic acid that is excreted in urine [19]. 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 [20, 21].

ALP level is increased in conditions such as 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 (1991) [22] where there were no significant in ALP level in CCl_4 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 celland therefore does not leak from damage cells into the blood as readily as soluble cytoplasmic enzyme [23].

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 ($p < 0.05$) in this protein that can be compared with that of the standard group. Nasir et al., [24] reported that there were significant decrease in serum total protein and albumin after administration of *A. paniculata* aqueous leaf extract in CCl_4 induced-hepatotoxicity in rats. The increase in serum protein and globulin in this study might be possibly due to the improvement of liver functions.

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.

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, Kamis et al. [25] found that at high concentration, the plant is hypolipidemic, hepatotoxic and nephrotoxic. However, Modu et al. [26] reported that aqueous pulp extract of H. 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.

3.1. Acute toxicity study (LD_{50}) of aqueous extract of Doum fruit in phase I and phase II (Lorke's method) [27]

Table 1. Results of acute toxicity study

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

- No death

3.2. Hepatoprotective activity study of aqueous extract of Doum fruit on paracetamol-induced liver injury albino rats

Table 2. Results of hepatoprotective study of *H. thebaica* in paracetamol-induced liver toxicity in rats

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

Values are expressed as mean ± SEM; b – statistically significant when compared to PCM at $p < 0.05$.

4. Conclusion

From the acute toxicity study, the LD_{50} of the extract was estimated to be greater than 5000 mg/kg. The aqueous extract of *H. thebaica* at different doses of 250 mg/kg and 500 mg/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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