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Decreased cardiovascular risk and resistance to hyperlipemia-induced hepatic damage in rats by aqueous extract of *Urtica dioica*

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Hyperlipemia and Hepatic metabolism was studied in hyperlipemic albino rats maintained on a high fat diet. Aqueous extract (100, 200, 300mg/kg/day) of *Urtica dioica* corrected dyslipidemia and restored hepatic chemistry in hyperlipemic animals. The extract was effective in normalizing the atherogenic lipoprotein phenotype. Total cholesterol (CHOL), Triglyceride (TG), Low density lipoprotein cholesterol (LDL), LDL/HDL-ratio, and Total Non-HDL cholesterol (TNH-CHOL) were significantly reduced by the treatment. There was no significant effect of treatment with extract on the high density lipoprotein cholesterol (HDL). Hyperlipemia was associated with significant elevations in serum liver enzymes (ALT, AST, LDH, and -GT) activities that are markers of altered hepatic chemistry. These elevations were however normalized by treatment with the extract. Hyperlipemia also induced a significant increase in bilirubin levels, decrease in total protein and decrease in albumin levels. These alterations in hepatic chemistry were normalized by treatment with *U. dioica* at aqueous extract concentration of 300 mg/kg. The study shows that aqueous extract of *U. dioica* may restore lipemic normalcy, and may posses a potential for reduction of cardiovascular risk and a resistance to hyperlipemia-induced hepatic damage in rats.

Keywords: *Urtica Dioica,* hyperlipemia, hepatic chemistry, cardiovascular risk, rats.

INTRODUCTION

The liver plays a significant role in the body as the organ saddled with the responsibility of metabolising toxic substances that enter the body. The major functions of the liver can be detrimentally altered by liver injury resulting from acute or chronic exposure to toxicants or by situations affecting both -oxidation and the respiratory chain enzymes. Altered free fatty acid metabolism and impaired aerobic respiration as found in animals on high fat diet, have frequently been associated with accumulation of lactate and reactive oxygen species (ROS). The presence of ROS further disrupts mitochondrial DNA and brings about the damage to hepatic cells. Patients with hyperlipemia have elevations in aminotransferases levels due to non-alcoholic fatty liver disease (Mayes et al., 2003). It is well known that hyperlipemia induces liver damage (Mukai, 2002; Milionis et al., 2004). Dyslipidemia, as low HDL-cholesterol, high triglyceride and elevated LDL-cholesterol, increases cardiovascular disease Risk (Wilson, 1991; Austin, 1998). LDL, HDL and TG are all independent and significant predictors of cardiovascular risk. High blood cholesterol is one of the greatest risk factors contributing to the prevalence and severity of coronary heart disease (Wilson, 1998**)**. The total non HDL cholesterol (TNH-CHOL) is the single greatest predictor of cardiovascular risk and can be used as a surrogate measure of lowering the cardiovascular risk. There is evidence that serum cholesterol levels in infancy correlate with mortality by coronary heart disease in the adulthood (Gordon, 1977) . In addition, there is a well known association between hypertension and dyslipidemia, particularly high levels of serum LDL cholesterol. Furthermore, the magnitude of the reduction in

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cardiovascular events is a function of the extent of LDL cholesterol lowering, with each decrease of 40 mg per deciliter (1.0mmol/l) in LDL cholesterol corresponding to a 24% reduction in major cardiovascular events (Baigent et al., 2005).

The adoption of crude extracts of plants, such as infusions, for self-medication by the general public (Houghton, 1995), has arisen in the possibility that the impact of several diseases may be either ameliorated or prevented by improving the dietary intake of natural nutrients (Haslam, 1996). *Urtica dioica* (Urticaceae), also known as Stinging Nettle, is a perennial herb indigenous to Italy and the Mediterranean regional countries and grows in nitrogen rich soils. The leaf of the plant *U. dioica* has been shown to have anti-inflammatory activity and *U. dioica* aqueous and to a lesser extent petroleum ether extract have been shown to improve the lipid profile in normolipidemic rats fed with a regular or a high-fat diet (Dehar et al., 2006). We are not aware of any studies on the hepatoprotective effect of *U. dioica* on high fat diet rat. The purpose of this study is to evaluate hypolipemic vis-à-vis hepatoprotective effects of *U. dioica* extracts in high fat diet hyperlipemic rats.

MATERIALS AND METHODS

Collection and identification of plant materials

Aerial part of the plant *U. dioica* was collected from Trans-Egbu, owerri west in Imo state, Nigeria. Identity of the plant was confirmed by Mr. J. M. C. Ekekwe a plant kingdom scientific analyst. A voucher specimen (Voucher No. 0031) was deposited in the authors laboratory

Extraction

Aqueous extracts were prepared according to the traditional method used in Nigeria (decoction): 200 g of powdered aerial plant dried at room temperature was mixed with 2000 ml of pre-boiled distilled water for 3 h under vigorous shaking, then decanted and filtered. Extract was concentrated under vacuo and freeze dried (Yield 12.4%). The dry extract was stored in a desiccator protected from light and moisture.

Animals used

Healthy, adult male albino Wister rats (200 – 240 g) were used in all experimental procedures. Animals were housed in stainless steel cages, acclimatized under standard environmental condition (27 ± 1 C, and a 12-h light/dark cycle) and maintained with free access to water and a standard rodent diet (proteins; 24%, carbohydrates; 50%, lipids; 11%, vitamins; 3%) adjusted with 1.5% cholesterol *ad libitum*. Animals were processed according to the suggested international ethical guidelines for the care of laboratory animals.

Protocol

Animals were randomly divided into 5 groups of 8 rats each. Group 1 was the Non-lipemic control group (NLC) and received normal diet and water. Groups 2, 3, 4, and 5 were the hyperlipemic groups that received standard diet adjusted with 1% cholesterol and 0.25% sodium cholate for 70 days to induce hyperlipemia following a modified scheme of Hyeung et al. (2006). Group 2 was the Hyperlipemic control (LC) and remained untreated while groups 3 (UDE 100), 4 (UDE200) and 5 (UDE300) received 100, 200, and 300 mg/kg/day of the *U. dioica* aqueous extracts respectively administered orally by gavage. Treatment was introduced on day 29 and lasted forty one (41) days.

Analyses of serum enzyme activities and lipid profiles of the blood samples

At the end of treatment, animals were fasted overnight (14 h) and sacrificed by cervical dislocation. Blood was collected by heart puncture. The blood samples of each animal were taken and allowed to clot for 45 min at room temperature. Serum was separated by centrifugation at 600×*g* for 15 min and analyzed for various biochemical parameters. Total cholesterol, triglyceride, HDL-cholesterol, and LDL-cholesterol levels were measured spectrophotometrically (Pharmacia LKB Ultospec III) using assay kits (Biosystems S.A. costa Brava Barcelona Spain) while LDL/HDL- ratio, and the total non-HDL cholesterol level (TNH-CHOL) were simply calculated. Serum enzyme activities (ALT, AST, gamma-glutamyl tranferase -GT, lactate dehydrogenase LDH, total protein, albumin and bilirubin assays were determined using a chemistry analyte (Ciba-Corning 550 Express Plus. USA).

Statistical analysis

Data were expressed as the mean ± S.D. The significance of the results was calculated using Student's t - test and the levels of statistical significance were set at $P \leq 0.05$.

RESULTS

Effect of aqueous extract of *U. dioica* on serum enzyme activities and bilirubin

Serum enzyme activities (ALT, AST, LDH and - GT) and bilirubin were significantly elevated in the lipemic animals when compared to the non- lipemic control (Table 1). Administration of the aqueous extracts of the plant at 100 mg/kg/day (UDE 100) significantly ($P \leq 0.05$) lowered the activity of ALT, AST and LDH when compared to the hypercontrol group. The activity of -GT and bilirubin were significantly lowered when compared to the hyper-control group after administrating the extract at 200 mg/kg/day, while at 300 mg/kg/day the activities of the serum enzymes and bilirubin were further reduced but were significantly ($p \leq 0.05$) higher than the non-lipemic control.

Effect of aqueous extract of *U. dioica* on the levels of serum total protein and albumin

Hyperlipemia produced a significant decrease in the serum total protein and albumin in the lipemic control animals compared to the non-lipemic control (Table 1). Administration of extract at 100 and 200 mg/kg/day did

Table 1. Effect of *Urtica dioica* aqueous extracts on some serum enzyme activities and on the levels of bilirubin, total protein and albumin.

	ALT (IU/L)	AST (IU/L)	LDH (IU/L)	-GT (IU/L)	Bilirubin (mg/dl)	Total protein (mg/dl)	Albumin (mg/dl)
NLC	b78±5.7	b185±7.2	b1281.1±47.3	b6.76±0.80	b9.4±1.1	b6.51±0.77	b4.19±0.29
LC	A116±4.1	a206.4±13.8	a1619.1±110.0	a15.49±1.91	a14.6±1.4	a5.81±0.44	b3.70±0.22
UDE 100	a b103±6.2	B193.5±9.4	b1199.4±100.3	a13.69±1.70	a13.4±1.2	5.90±0.53	b3.86±0.24
UDE 200	a,b93±8.6	b184.4±5.9	a,b1000.6±76.3	a,b11.28±0.96	a,b11.9±1.1	6.23±0.43	3.93±0.30
DE 300	b81±7.3	b179±4.7	a,b902.5±118.2	a,b8.43±0.69	a,b10.9±0.9	b6.36±0.49	b4.03±0.17

Values = Mean ± Standard deviation (n = 8)
a P< 0.05 Significant difference with respect to the non lipemic control (NLC)
b P< 0.05 Significant difference with respect to the lipemic control (LC).

not produce a significant increase in the levels of total protein and albumin compared to the lipemic control group. The extract at 300 mg/kg/day however produced a significant increase compared to the lipemic control group, which was comparable to the non-lipemic control group.

Effect of aqueous extract of *U. dioica* on serum lipid profile

Total cholesterol (CHOL), triglyceride (TG), LDL, LDL/HDL-ratio and total non-HDL cholesterol (TNH-CHOL) were all significantly reduced by the treatment compared to the lipemic control group (Figure 1) . There was no significant ($p < 0.05$) effect of treatment on the high density lipoprotein cholesterol (HDL) compared to the lipemic control group.

DISCUSSION

Serum enzyme activities are used as indicators of chemically induced liver damage (Drotman et al., 1978). Injuries to the liver associated with marked alteration in liver chemistry have variously been treated using crude extracts of plants (Raja et al., 2007, Bhandarkar et al., 2004). Hepatotoxicity has been viewed as liver injury associated with impaired liver function caused by exposure to drug or other non-infectious agents (Navarro, 2006). It is well known that hyperlipemia induces liver damage (Mukai et al., 2002). Our model of hyperlipemia was accompanied with altered hepatic chemistry as showen by the elevations in serum ALT, AST, LDH and -GT activities in the hyperlipemic controls. These elevations were not however comparable to levels found in most drug hepatotoxicity (more than three times higher than the upper limit of normal ALT range, or more than two times higher than the upper limit of normal the total bilirubin level) (Temple, 1990; Lee, 2005). Hepatotoxicity induced in our model could be described as mild to moderate. The reduction in the serum liver enzyme activities in the UDE100, UDE200, and UDE300 groups indicates that *U. dioica* plant extract may protect against liver injury. Data (Table 1) showed that hyperlipemia induced increases in the total bilirubin, decreases in the serum total protein and decreases in the serum albumin. This indicates that impairment of liver function accompanied the injury caused by hyperlipemia. It is known that most circulating proteins are synthesized in the liver and levels indicate synthetic capability of the liver. Serum albumin accounts for about 65% of serum proteins (Deepak et al., 2000). Altered serum protein levels are common in liver disease but are nonspecific (Friedman et al., 1996; Martin et al., 1998). The extract at 300 mg/kg/day was able to ameliorate injury and restore functions. This was evidenced in the restoration of the total protein and albumin to levels not significantly ($p \leq 0.05$) different from the non-lipemic controls. The distinction between injury and function is important, because it is mainly when function is impaired that symptoms and clinically signify-cant disease follow (Navarro, 2006).

The plant extract was highly effective in normalizing the atherogenic lipoprotein phenotype in agreement with the findings of Dehar et al. (2006). The reduction in the levels of CHOL, TG, LDL TNH – CHOL and LDL/HDL in the presence of the aqueous *U. dioica* extracts points to a potential of the extract to reduced cardiovascular disease risk. It is well known that these indices are all independent and significant predictors of cardiovascular disease risk (Wilson, 1998). The reduction in the TNH-CHOL is most interesting since the total non-HDL cholesterol (TNH-CHOL) is the single greatest predictor of cardiovascular risk. TNH-CHOL has been shown to be as good as or better than apolipoprotein fractions in the prediction of cardiovascular risk (Paul et al., 2005). Administration of the extract up to 300 mg/kg/day throughout the study did not however increase the HDL values in the treated groups compared to the lipemic control group.

The study shows that aqueous extract of *U. dioica* has a hypolipemic effect ipso facto a potential for reduction of cardiovascular risk and a resistance to hyperlipemia- induced hepatic damage in rats.

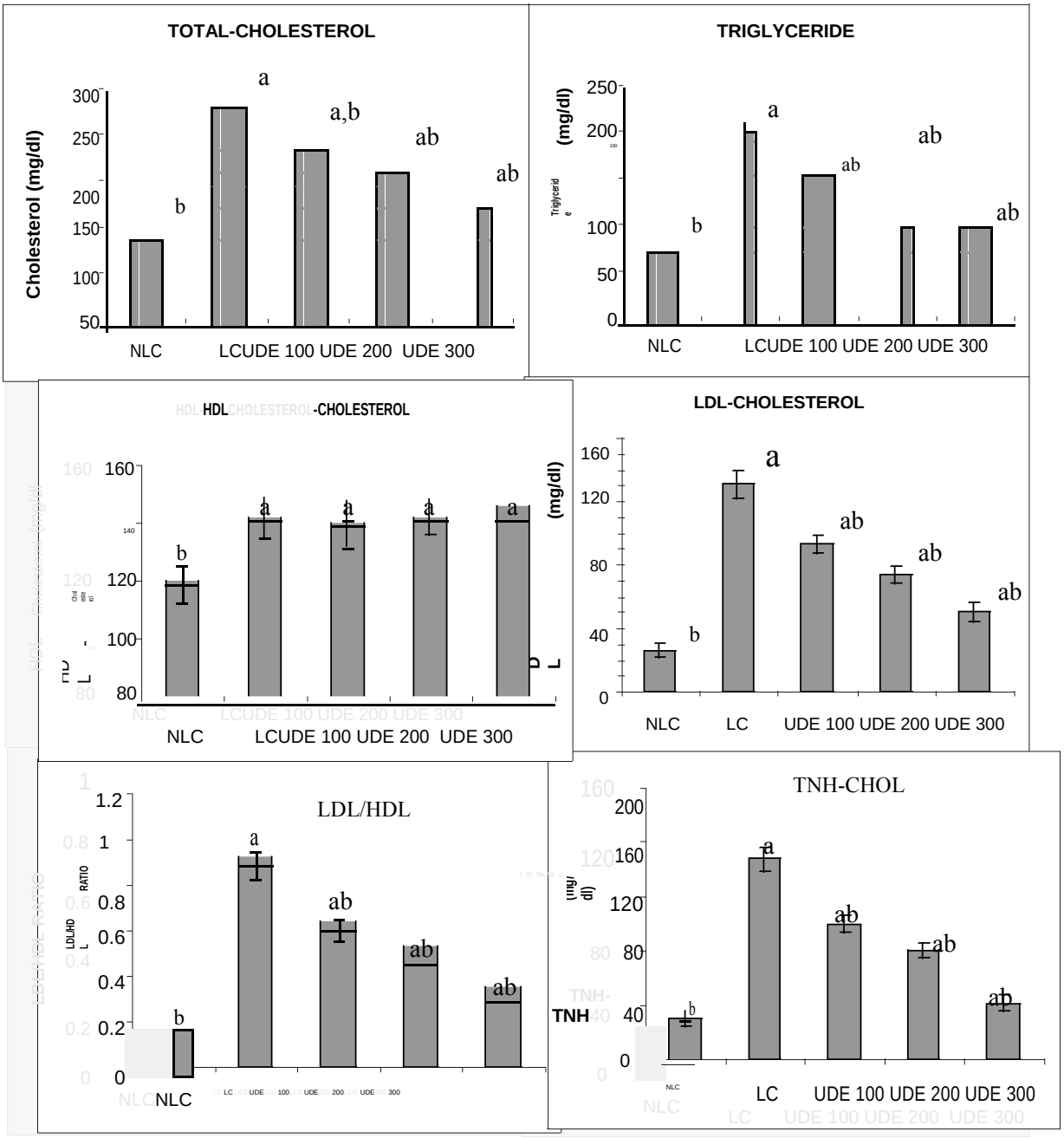

Figure 1. Effect of aqueous extract of *Urtica Dioica* on the serum lipid profile in hyperlipemic rats after a chronic dosing 49 days treatment.
Values = mean ± standard deviation (n = 8),
a P< 0.05 Significant difference with respect to the non lipemic control (NLC)
b P< 0.05 Significant difference with respect to the lipemic control (LC)

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