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Effects of Ethanol Extract of *Acalypha torta* Leaves on the Lipid Profile and Serum Electrolytes of Rabbits

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ABSTRACT

The effects of ethanol extract of *Acalypha torta* leaves on the lipid profile and serum electrolytes of rabbits after a three-week treatment was investigated. Findings show that treatment of the animals with 10.0 and 20.0 mg/kg body weight of extract caused significant ($p < 0.0001$) reductions in the concentrations of serum triacylglycerol, total cholesterol and LDL – cholesterol within 14days of commencement of treatment. On the other hand, HDL-cholesterol concentration was increased significantly ($p<0.001$). Significant increases ($p < 0.001$) in LDL – cholesterol concentrations were also observed in all the treated groups of rabbits after the 14th day whereas HDL-cholesterol decreased. The concentrations of serum electrolytes (Na^+, K^+, CL^- and HCO_3^-) were not affected to any significant extent.

Keywords: Hypertension, atherosclerosis, hyperlipidaemia, *Acalypha torta,* hypocholesterolaemia

INTRODUCTION

According to the low-density-lipoprotein (LDL) receptor hypothesis, development of atherosclerosis is caused by a high concentration of LDL-cholesterol in the blood. Lowering LDL-cholesterol concentration therefore reverses, or at least retards the onset of atherosclerosis, thus preventing cardiovascular disease. Research findings have proved that lowering the concentrations of plasma lipids could diminish the complications of atherosclerosis and hypertension thereby prolonging life (Brown and Goldstein, 1992; Ostland, 2000).

Herbal remedies are becoming increasingly popular and scientific evidence of efficacy of these herbs is beginning to emerge from controlled preclinical and clinical trials. And of course, a number of commonly used pharmaceutical products are of botanical origin. Aspirin, digitoxin, quinine and reserpine are well known examples (Aschwanden, 2001). The leaf extract of *Acalypha torta* Muell (English: Copper or Jacob's coat) has been alleged to possess diverse medicinal properties. In Nigeria, for instance, the leaves are used for the treatment of malaria, rheumatism, stomach upset, dermatitis,and infantile eczema (Irobi and Banso, 1994).

The efficacy of *Acalypha torta* Muell leaf extract as an antihypertensive agent was demonstrated by Ezekwesili (2007). The aim of the study was to investigate the effects of the crude ethanol leaf extract on serum lipids and electrolytes since these biochemical parameters have been found to correlate positively with the incidence of hypertension (Peggy and George,1983;Khaw and Barretto-Conor,1990;Coruzzi *et al*.,2001).

MATERIALS AND METHODS

Plant Materials

Mature *Acalypha torta* Muell leaves were collected from Abagana, Anambra State, Nigeria and the plant identified in the Department of Botany, University of Nigeria, Nsukka. Voucher specimen was prepared and deposited in the International Centre for Ethnomedicine and Drug Development (INTERCEDD) herbarium with No.8256.

Experimental Animal Models

Twelve adult healthy male albino rabbits weighing between 1.9 and 2.5 kg were purchased from the main market in Awka, Anambra State, Nigeria. The animals were kept in the animal house of Department of Biochemistry for two-weeks to allow for acclimatization.

Chemicals

Chemicals and reagents used included ethanol, chloroform and methanol from BDH England; triacylglycerol, cholesterol, LDL-cholesterol and HDL-cholesterol diagnostic kits were obtained from Biosystems S.A. Costa Brava, Spain. All other reagents used were of analytical grade.

Methods

Extraction Procedure

Four hundred grams of dried and pulverized leaves of *Acalypha torta* were soaked in 2.0 litres of chloroform-methanol (2:1) at room temperature for 72h. Three changes of solvent were made at 24h intervals. The extract was filtered through cheesecloth and Whatman no.1 filter paper and the filtrate was discarded. The residue from chloroform-methanol (2:1) extraction was then dried

the filtrate was discarded. The residue from chloroform-methanol (2:1) extraction was then dried and re-extracted thrice in 2.0 l of ethanol at room temperature and at 24h intervals. After filtration, the filtrate was evaporated to obtain the thick brown slurry which was refrigerated and used as the crude ethanol extract.

Lipid profile and electrolyte studies.

Twelve adult healthy male rabbits were weighed and divided into three groups of four rabbits each according to their body weights. They were maintained on standard diet of growers mash (Guinea Animal Feed) and vegetables. Access to drinkable water was allowed *ad libitum* throughout the period of study. The control group of rabbits (T_0) received a daily dose of normal saline, 1.0 ml/kg body weight (b.wt) of animals, whereas two different doses of the ethanol extract of *Acalypha torta*, 10.0 and 20.0 mg/kg b. wt were administered daily to the test groups (T_1 and T_2) respectively. All administrations were intraperitoneally for twenty-one days. Blood samples were collected via the ear lobes of the animals on days 1, 7, 14 and 21.

After clotting, the blood samples were centrifuged at 3,000 rpm and the supernatant sera samples were used for lipid profile and electrolyte concentration estimations.

Determination of Serum Cholesterol Concentration

Total serum cholesterol was determined according to the method of Richmond (1973) using Biosystems Diagnostic Kit.

Determination of Serum Triacylglycerol Concentration.

The quantitative method of Bucalo and David (1973) was used to determine the triacylglycerol concentrations of the experimental animals. Biosystems Diagnostic Kit was used.

Determination of Serum LDL-cholesterol concentration

Assman's (1984) method of determination was employed using Biosystems Diagnostic Kit for LDL-cholesterol estimation.

Determination of Serum HDL – cholesterol concentration

Groves' (1979) method for HDL-cholesterol estimation was adopted. Biosystems Diagnostic Kit was used for the experiments.

Determination of Serum Potassium and Sodium Ion Concentrations.

Serum concentrations of these ions were determined using flame photometry according to AOAC (1984).

Determination of Serum Chloride Ion Concentration.

The method of estimation was that of Schoenfield and Loewell (1964).

Determination of Serum Bicarbonate Concentration.

Titrimetric method by Vanslyke and Cullen (1917) was adopted.

Statistical Analysis.

Statistical analyses of all the data were carried out using ANOVA and Bonferroni's Multiple Comparison Test. Significant differences were accepted at $p < 0.05$. Data were expressed as means $\pm$ standard deviation.

RESULTS

Effect on lipid profile

In the analyses, values obtained from the control group of rabbits were compared with values recorded for the groups treated with the extract of *Acalypha torta*, while variations between groups treated with different doses of the extract were analysed to establish the influence of dose on the activity of the extract. In all the figures, values recorded on day 1 before treatment commenced were regarded as the baseline values.

Fig. 1 shows that up till day 14, the extract had no effect on the serum triacylglycerol concentrations in all the test groups when compared with control rabbits. On the other hand, by day 21, 10.0 mg/kg. b.wt. of *A. torta* extract caused 23.5% (ie from 7.57 $\pm$ 0.230 to 5.79 $\pm$ 0.010) reduction in the concentration of tricylglycerol whereas significant ($p < 0.001$) decrease 35.2% (4.35 $\pm$ 0.012 to 2.82 $\pm$ 0.007) in triacylglycerol concentration was obtained at a higher dose, 20.0 mg/kg. b.wt. of the extract. This observation would indicate that the effect of the extract was dose - dependent.

Both dosage forms caused slight but insignificant decrease in the concentration of serum cholesterol up to day 7 (Fig 2). Findings recorded on day 14 showed that daily administration of 10.0 mg/kg b.wt of extract depressed total cholesterol concentration by 19.2% (i.e. from 39.90 $\pm$ 0.003 to 32.06 $\pm$ 0.016). There was no substantial difference in the magnitude of decreases obtained on days 14 and 21 (19.2% and 20.5% respectively. But significant ($p < 0.001$) reduction of 53.31% (from 58.32 $\pm$ 0.006 to 27.23 $\pm$ 0.10) was observed with 20.0 mg/kg. b.wt dose of extract. This also points to the fact that the effect of extract was dose – dependent.

The extracts (10.0 mg/kg b. wt. and 20.0 mg/kg. b. wt.) produced significant decreases ($p < 0.001$)

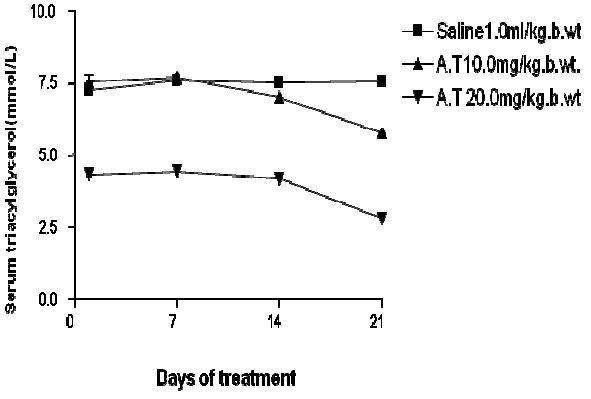

Fig1: Effect of ethanol extract of *A.torta* leaves on serum triacylglycerol concentrations

increased HDL-cholesterol concentration from initial value of 3.10 ± 0.076 to 5.09 ± 0.053 (i.e 64.2%) whereas 20.0 mg/kg b. wt. produced a larger increase in serum HDL-cholesterol from 2.02 ± 0.003 to 5.19± 0.009 (i.e 156.93%).There were no observable differences in the concentration of HDL-cholesterol in the test groups of rabbits beyond day 14. By day 21, the extracts, at 10.0 mg/kg b. wt and 20.0 mg/kg b. wt significantly ($p < 0.0001$) reduced HDL-cholesterol by 61.9% (3.10 ± 0.076 to 1.80 ± 0.10) and 70.30% (2.02 ± 0.003 to 0.60 ± 0.001) respectively.

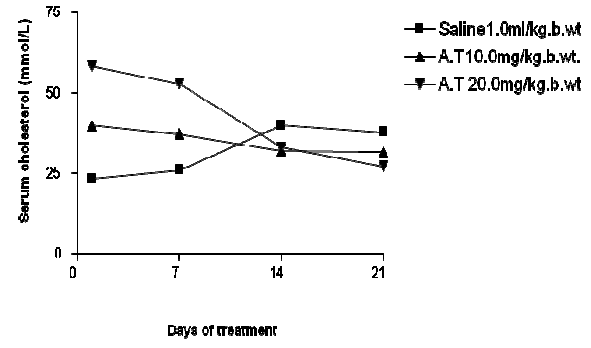

Fig 2: Effect of ethanol extract of *A. torta* leaves on serum total cholesterol concentrations

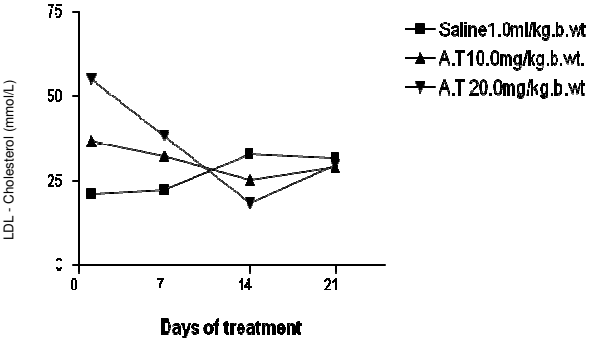

Fig3: Effect of ethanol extract of *A. torta* leaves on LDL-cholesterol concentrations

Effects On Serum Electrolytes

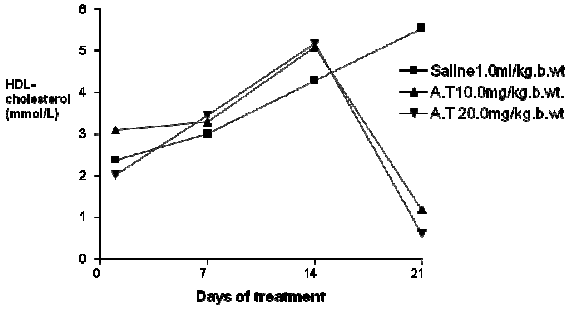

Fig4: Effect of ethanol extract of *A. torta* leaves on HDL-cholesterol concentrations

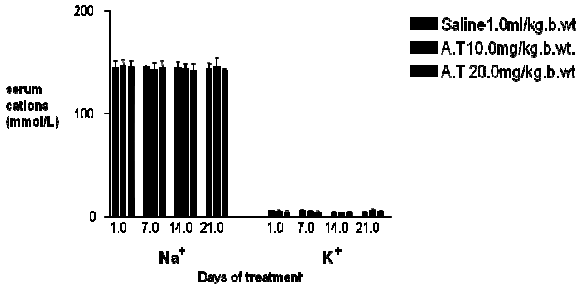

Fig 5:Serum cation concentrations after treatment with ethanol extract of *A. torta* leaves

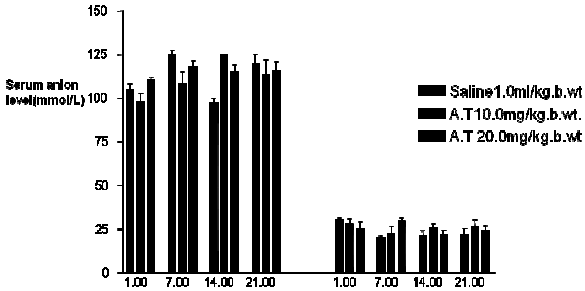

Fig 6: Serum anion concentrations after treatment with ethanol extract of leaves of *A. torta*

Crude leaf extract of *Acalypha torta*, at both doses (10.0 and 20.0 mg/kg b. wt.) used did not have any significant effect ($p > 0.05$) on the principal serum cations (Na^+ and K^+). On the other hand, increases of 14.38% and 15.46% in CL^- concentrations were recorded at 10.0 and 20.0 mg/kg b. wt. respectively ($p > 0.05$) compared to values obtained for control animals. Serum bicarbonate ion concentrations were not affected (figs 5 and 6).

DISCUSSION

In recent times, there has been a decline in the prevalence of artherosclerosis and artherosclerosis – related deaths possibly due to effective management of the risk factors that predispose to this disorder. The major identified risk factors are elevated LDL-cholesterol, reduced HDL-cholesterol (Ghasi *et al*, 2000; Massing *et al*,

2001) hypertension and non-insulin dependent diabetes mellitus (Chattopadhyay and Bandyopadhyay, 2005). Lowering of serum lipid concentrations, particularly LDL and VLDL fractions, is therefore considered as one of the strategies that can delay the on-set of chronic disorders associated with hyperlipidemia in humans.

Herbal extracts are often used in folk medicine to improve the lipid profile in humans (Chattopadhyay and Bandyopdhyay, 2005).In this study, results obtained on the time course of the effect of ethanol leaf extract of *Acalypha torta* on the lipid profile of normal rabbits have revealed that this plant could improve the lipid profile, particularly total cholesterol and LDL-cholesterol, between the 7^{th} and 14^{th} day of daily treatment. This was deduced from the fact that the serum concentrations of these fractions in the treated animals were significantly ($p<0.0001$)) lower, on day 14 compared to the baseline values (day 1) and control animals. On the other hand, HDL-cholesterol concentration was significantly ($p<0.001$) raised. Plasma clearance of LDL particles is mediated primarily by LDL receptors, a large complement of which is expressed by the liver (Dietschy *et al*, 1993) and LDL become atherogenic when they are modified by oxidation reaction (Steinberg, 1997). *Acalypha torta* leaf extract might be inducing rapid catabolism of low -density lipoprotein cholesterol through hepatic receptors for final elimination in the form of bile acids. This mechanism is yet to be experimentally investigated.

Plant sterols have been reported to lower cholesterol by lowering plasma concentration of LDL (Law, 2000; Ostlund, 2002). Chemical analyses revealed that *Acalypha* species contain high concentrarions of polyphenols, terpenoids, and plant sterols (Adesina *et al*, 2003; Ezekwesili, 2007). These bioactive phytochemicals are presumed to be wholly or partly responsible for the lipid- lowering action of *Acalypha torta* extract.

The *Acalypha* species are well known for their antimicrobial activities (Irobi and Banso, 1994; Adesina *et al*. 2000; Ogundaini, 2005; Oladunmoye, 2006). Oladunmoye (2006) reported alteration of serum sodium and potassium ion concentrations as the basis for the antimicrobial activity of Acalypha wilkesiana. In this study, *Acalypha torta* extract had no statistically significant effect ($p>0.05$) on serum concentrations of the electrolytes (Na^+ and K^+).This implies that the extract does not interfere with the sodium and potassium pumps or Na^+ / K^+ ATPases which are among the metabolically very important pumps.

Thus, *Acalypha torta* leaves may be considered beneficial in the treatment of hyperlipidaemia and its associated complications such as hypertension and atherosclerosis. But prolonged use of the extract may not be beneficial since the extract was more potent on the 14^{th} day than on the 21^{st} day of treatment.

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