REDUCTION OF RAT SERUM AND HEPATIC PHOSPHOLIPID LEVELS BY AQUEOUS EXTRACTS OF
GONGRONEMA LATIFOLIUM.

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Summary
The fasting serum and hepatic phospholipid levels were determined in rats treated to 27.77mg and 54.54mg/kg body weight oral doses of the aqueous extracts of Gongronema latifolium for fourteen days. The extract-treated rats showed significantly lower (P < 0.05) levels of both serum (3–63%) and hepatic (20–77%) phospholipid relative to the pretreatment levels of the lipid. The effects of the extracts are concentration dependent and while the effects of lower concentrations (< 54.54mg/kg) of the extracts could be relieved within the experimental period, the effects of higher concentrations (> 54.54mg/kg) of the extract could be prolonged and may elicit adverse effects on the liver phospholipid metabolic pattern. It was suggested that these results could imply that prolonged use of the plant leaves may adversely affect the health of the consumers.

Key words: Serum hepatic phospholipid rat Gongronema latifolium.

Introduction
Gongronema latifolium, Benth (Asclepiadaceae) formerly known as Marsdenia latifolia R. Schum, is a fast growing, shade-loving, perennial climbing or creeping plant. The stem is soft and pliable, producing Milky latex when pricked. It grows mostly in the tropical forest and compound farms (Nelson, 1965; Okafor, 1990; Burkill 1985). It is widely used in the West African sub-region for a number of medicinal and nutritional purposes (Dalziel, 1937). In the Southern parts of Nigeria, the plant leaves are used as vegetables and as spice in meat and salad preparations. It is also used as a base for cooking of food served to the sick. Okafor (1980) reported that the aqueous extracts of the plant leaves are used for treatment of loss of appetite, cough and stomach ache. In Ghana, the boiled fruits are used as a laxative, while in Sierra Leone, a decoction of cold infusion of the pounded stem is used for colic

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and intestinal symptoms associated with worms (Dieghton, 1957). Additionally, the leaves when used as spice for the soup of nursing mothers, are believed to serve as stimulants for rapid contraction of the womb and the return to menstrual cycle.

The pleasant bitter taste conferred on food by the plant leaves makes the leaves very attractive to its users. Phytochemical studies on the aqueous extracts of the leaves reveal the presence of a variety of bioactive compounds including alkaloids, tannins, saponins, flavonoids etc. (Obasi and Okoro 1997) Gamaniel and Akah, (1996). Bitter principles in plants have been shown to be alkaloidal (Kupchan 1971) and this class of compounds could be responsible for the bitter taste of Gongronema latifolium leaves. Additionally, the variety of compounds in the various classes of bioactive compounds shown to be present in the aqueous extracts of the plant leaves, implies that the pharmacological effects of the extract could be varied. Consequently, because of the extent of the use of the plant leaves for medicinal and nutritional purposes within the West African subregion including our locality, we are carrying out systematic investigations into the pharmacological effects of the crude aqueous extracts of the plant leaves in laboratory animals. This is with a view to ascertaining the effects of the extracts on key metabolic processes of the animals. Findings from these investigations could be useful in predicting the possible association of the use of the plant materials in the treatment and incidence of endemic metabolic disease states.

In the present investigation, we studied the effects of the crude aqueous extracts of the leaves on rat serum and hepatic phospholipid levels. Studies on the effects of the crude aqueous extracts on serum and hepatic lipid levels were motivated by the need to study the possible association of the plant materials used in nutrition and ethnomedicine with the incidence of diseases related to lipid metabolism.

Materials and methods

Plant Materials

Fresh leaves of Gongronema latifolium were collected from Uturu area of Abia State Nigeria.

Botany identification was confirmed in the Botany Unit of the School of Biological Sciences, Abia State University Uturu. A voucher specimen of the plant is kept in the School herbarium.

Chemicals and Reagents

The common reagents and chemicals used in these experiments were of analytical grade. Ammonium thiocyanate, chloroform and methanol were from BDH (Poole, England); anhydrous sodium sulphate, lecithins and ferric chloride hexahydrate were from Sigma Co. (St. Louis USA). Ammonium ferrothiocyanate was prepared fresh by mixing 27.03g of ferric chloride and 30.4g of ammonium thiocyanate in 1 litre of distilled water.

Preparation of Aqueous Extracts

The leaves were air dried and milled to coarse powder. The resultant powder was soaked (150g) in 800ml distilled water and left to stand for 24h with occasional shaking. Thereafter, the mixture was filtered and the filtrate contained 60.0 ± 1.5mg/ml of solid residue (crude extract).

Animal Treatment

Thirty-six male albino rats (Wistar Strain) of mean weight 144.16 ± 10.40gm were used. They were housed in stainless steel cages on raised platform in the animal house at normal tropical conditions (temperature = 28 ± 2°C, relative humidity = 70–90%). The animals were randomly distributed into three groups (A–C) consisting of 12 animals per group. All the animals were fed standard Top Feeds (Pfizer PLC. Lagos) containing 70.5% carbohydrate, 24.5% crude protein, 1.0% oil, 3% salt mix and 1% vitamin mix and water libitum, throughout the period of experiments. After a 7 day acclimatization period, 3 animals were randomly taken from each of the 3 groups for the determination of the pretreatment (or baseline) phospholipid levels of each of the animal groups. Thereafter, the animals in group B were orally treated with 27.77mg/kg body weight of the extract, while the group C animals were treated with 54.54mg/kg body weight of the extract orally. The doses represent half the LD₅₀, and the LD₅₀ values respectively, of the extract in mice as described previously (Obasi and Okoro 1997).

The animals in group A were orally treated...
to water (vehicle for the extract) in equivalent volume (1.0ml) with the extracts given to the test animals. The treatments were repeated each day for fourteen days. On the 14th day (last day of treatment), 19th and 24th days (from the first day of treatment) corresponding to the 0, 5th and 10th days from the last day of treatment respectively, 3 animals were taken from each of the animal groups for phospholipid level determination. In estimating the phospholipid levels of the animals at each period (including the animals taken for the determination of the pretreatment phospholipid levels), the animals were first fasted for 12h. After fasting, each of the animals was sacrificed by stunning and blood collected by cardiac puncture. The liver of each animal was also collected. Serum and hepatic phospholipid levels were determined by the method of Stewart (1980). This essentially involved mixing 0.1ml serum with 2.0ml chloroform-methanol (2:1) solution. The mixture was vortexed for 3–5 min to ensure thorough mixing. On phase separation, 1.0ml of the lower layer was further mixed with 2.0ml of ammonium ferrothiocyanate and further vorted for 3mins. On phase separation,

Results

The data in Tables 1 and 2 show that the fasting serum and hepatic phospholipid levels of the experimental (Groups B and C) rats were reduced when the levels of the lipid at the different periods after treatment, were compared with the pre-treatment levels of the lipid in each of the groups. These reductions were generally significant (P < 0.05). In the two experimental groups, the values of the percentage decrease was highest on the 14th/0 day periods from the first/last day of the experiment (Figs 1 and 2). The values of the percentage decrease reduced

Fig 1: Histogram of the percentage change (relative to pretreatment values) in the serum phospholipid levels of the different groups of rats at the different day periods from the first/last day of treatment. Plotted values were calculated from the values in Table I and multiplied by -1.

Fig 1: Histogram of the percentage change (relative to pretreatment values) in the hepatic phospholipid levels of the different groups of rats at the different day periods from the first/last day of treatment. Plotted values were calculated from the values in Table II and multiplied by -1.
groups after the treatments, were generally significant (P < 0.05) when compared with the corresponding values in the control group (Tables 1 and 2).

The plot in Fig. 1 indicates that the effects of 27.77mg/kg body wt dose on the serum lipid level were almost eliminated by the 24th (10th) day period. This was however, not so with the 54.54mg/kg body wt dose (i.e. double dose) of the extract. On the other hand, the effects of both doses of the extracts on the hepatic phospholipid levels were still significant up to the 24th (10th) day period (Fig. 2). The pattern of the plots in Figs. 1 and 2 indicate that the effects of the extract on the phospholipid levels of the rat were generally dose dependent.

Discussion

This investigation shows that the aqueous extract of *Gongronema latifolium* leaf elicits reduction in both the serum and hepatic phospholipid levels of rats. We had earlier observed (unpublished manuscript) that the extract increased the serum cholesterol, but decreased the serum triglyceride levels of rabbits. Reduced serum and hepatic phospholipid levels are reported in aggravated forms of acute hepatitis, fatty degeneration of the liver and thryotoxicosis (Strayer and Makarova 1989; Friedman et al. 1967). Consequently, the results of the present investigation could indicate incidence of diseased liver elicited by the extract in the experimental animals. This could lead to alteration of hepatic functions and a modification of the metabolism of lipids in the hepatocyte of the animals.

The effects of the extract were reduced with time, indicating the metabolic elimination of the active principles of the extracts from the animals’ body. Therefore, the effects elicited by lower concentrations (< 54.54mg/kg) of the extract could be relieved within the experimental period i.e. 10 days from the last dose treatment (Figs. 1 and 2). However, the pattern of the plots in the figures also shows that higher concentrations of the extracts could predispose to prolonged hypophospholipidaemia in the animals. This implies that the extract at 54.54mg/kg body wt. concentrations could be hepatotoxic, (Friedman et al. 1967) and may adversely affect the metabolism of phospholipids in the liver of rats. Since phospholipids are important biological molecules with a variety of functions in the animal system (Onyeneke, 1984), reduction in the levels of the lipids could have negative effects on the membrane lipids turnover and may predispose the animal to some lipidaemic disorders and associated pathological conditions. This would have far-reaching adverse implications on the health of the human consumers of the plant leaves, if rat to man extrapolation is acceptable.

The aqueous extract of *Gongronema latifolium* had been shown (Gamaniel and Akah, 1995) to contain such compounds as flavonoids, alkaloids and saponins. The diversity in the chemical nature and biological effects of these compounds could lead to a diversity in the effects of the crude extracts in animal system. This is with particular reference to the therapeutic effects reported by the local users, vis-a-vis the toxicological potentials of the crude extracts in experimental animals.

In line with these observations, further studies on the effects of the crude extracts on some key hepatic enzymes and the general pharmacological effects of the chemical constituents of the extract would be needed to elucidate the exact mechanism of the effects and safety of these extracts to its consumers.

References


