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Effect of liquid extract from fresh *Abutilon indicum* leaves and *Allium cepa* bulbs on paracetamol and carbontetrachloride induced hepatotoxicity

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In traditional medicine, herbal drugs are being used in the treatment of liver disorders. Among those are the leaves of *Abutilon indicum* commonly known as 'thuthi' [1]. The bulbs of *Allium cepa* are used as antiperiodic, antibacterial, aphrodisiac, diuretic and also in the treatment of jaundice, asthma, malarial fever and skin diseases [2–4]. The liquid extracts from fresh *Abutilon indicum* leaves and *Allium cepa* bulbs are mixed in equal proportion and used as a remedy against liver disorders in Madurai folk medicine. To determine whether there is a scientific basis for this popular use or not, the effects of the combined liquid extracts were assessed on paracetamol and carbontetrachloride-induced hepatotoxicities in rats.

Both paracetamol and carbontetrachloride produced marked liver damage at the doses used, as expected [5–7]. This was evidenced by enzymatic changes. The liquid extract exhibited good hepatoprotective activity (Tables 1, 2). It significantly reduced carbontetrachloride and paracetamol elevated serum levels of SGOT, SGPT, ALKP and bilirubin. The protection against carbontetrachloride-induced liver damage suggests a normalization of increased lipid peroxidation [8] and free radical scavenging action [9]. Enzymatic activation of carbontetrachloride by the CCl_3 free radical, disrupts the structure and function of lipid and protein macromolecules in the cell organelles and induces microsomal lipid peroxidation [8]. Hepatoprotective activity may be due to the inhibitory action of liquid extract on cytochrome P_{450} [9]. The leaves and bulbs of *A. indicum* and *A. cepa* are reported to possess anti-inflammatory, hepatopathy and antimicrobial properties which may also contribute to its hepatoprotective action [1–4]. Further studies are in progress to isolate constituents and also to evaluate the exact mechanism of action.

Experimental

The leaves of *A. indicum* used in this study (collected from Madurai, Tamil Nadu) and *A. cepa* bulbs (from the local market) were identified by Dr. S. Jeyaselan in the Botany Section of Ayya Nadar Janaki Ammal College, Sivakasi, Tamil Nadu (India). The fresh leaves of *A. indicum* and *A. cepa* were crushed without using water; the expressed, palatable dense liquid extracts so obtained were carefully filtered and mixed in equal proportions and used for the studies. Wistar albino rats (150–200 g) of either sex maintained under standard animal housing conditions (12 h light and dark cycle) were used for all sets of experiments comprising of eight rats each. The rats were allowed to take standard laboratory feed and water ad libitum. The effects of a combined liquid extract on paracetamol and carbontetrachloride-induced hepatotoxicities in rats were assessed. In the paracetamol-induced hepatotoxicity model [10], the liquid extract (1 ml and 2 ml/kg) and silymarin (100 mg/kg) were administered orally to respective groups once daily for 3 days. On the 3rd day of treatment, paracetamol (3 g/kg body weight) was administered 30 min after administration of the test suspensions; control animals received just vehicle. 48 h after paracetamol administration, blood was collected from all groups of rats by puncturing retroorbital plexus. Serum was separated by centrifugation at 2500 rpm at 37 °C for 15 min and analyzed for various biochemical parameters, i.e. serum glutamic oxaloacetate (SGOT) [11] serum glutamic pyruvate transaminase (SGPT) [11] alkaline phosphate (ALKP) [12] total bilirubin (T. Bil) [13] and direct bilirubin (D. Bil) [13] according to the reported method to assess liver function.

In the carbontetrachloride-induced hepatotoxicity model [14], the liquid extract (1 ml and 2 ml/kg) and silymarin (100 mg/kg) were administered orally to respective groups three times at 12 h intervals; control animals received just vehicle. Carbontetrachloride diluted with liquid paraffin (1 : 1) was administered in a dose of 1 ml/kg body weight for 2 days. After 36 h of carbontetrachloride treatment, blood was collected. Serum was separated and analyzed for various biochemical parameters as in the case of paracetamol induced liver damage. The mean value ± SEM was calculated for each parameter. Results were statistically analyzed by Student's 't' test [15]. $p < 0.01$ indicates significant differences between group means.

Table 1: Effect of liquid extract from *Abutilon indicum* leaves and *Allium cepa* bulbs on paracetamol-induced hepatotoxicity in rats

Design of treatment	SGOT	SGPT	ALKP	T. Bil	D. Bil
Control	112.69 ± 6.18	48.06 ± 2.63	122.36 ± 6.13	1.04 ± 0.16	0.23 ± 0.02
Paracetamol	376.22 ± 3.0[a]	294.28 ± 3.18[a]	338.31 ± 1.50[a]	3.66 ± 0.20[a]	0.80 ± 0.04[a]
Silymarin	118.66 ± 12.34[b]	46.08 ± 1.18[b]	93.21 ± 3.72[b]	1.07 ± 0.08[b]	0.26 ± 0.01[b]
Liquid extract (1 ml/kg)	263.22 ± 7.20[b]	87.44 ± 4.22[b]	133.22 ± 3.18[b]	1.44 ± 0.14[b]	0.38 ± 0.02[b]
Liquid extract (2 ml/kg)	212.21 ± 8.24[b]	83.82 ± 5.23[b]	115.13 ± 3.21[b]	1.24 ± 0.12[b]	0.32 ± 0.02[b]

Values are mean ± S.E.; n = 8; a – $p < 0.01$ compared to control; b – $p < 0.01$ compared to paracetamol

Table 2: Effect of liquid extract from *Abutilon indicum* leaves and *Allium cepa* bulbs on carbontetrachloride-induced hepatotoxicity in rats

Design of treatment	SGOT	SGPT	ALKP	T. Bil	D. Bil
Control	127.43 ± 2.33	62.16 ± 1.78	139.74 ± 6.21	1.01 ± 0.20	0.18 ± 0.01
Carbontetrachloride	823.45 ± 7.31[a]	690.27 ± 8.20[a]	438.61 ± 8.21[a]	3.66 ± 0.38[a]	1.60 ± 0.30[a]
Silymarin	152.22 ± 3.10[b]	65.34 ± 2.00[b]	163.81 ± 4.28[b]	0.96 ± 0.05[b]	0.24 ± 0.02[b]
Liquid extract (1 ml/kg)	353.24 ± 7.33[b]	263.25 ± 4.12[b]	238.43 ± 8.33[b]	1.61 ± 0.31[b]	0.42 ± 0.02[b]
Liquid extract (2 ml/kg)	273.31 ± 7.21[b]	202.33 ± 4.08[b]	199.31 ± 8.17[b]	1.34 ± 0.42[b]	0.33 ± 0.02[b]

Values are mean ± S.E.; n = 8; a – $p < 0.01$ compared to control; b – $p < 0.01$ compared to carbontetrachloride

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Aframodial, a labdane diterpene showing selective *in vitro* antileukemic activity

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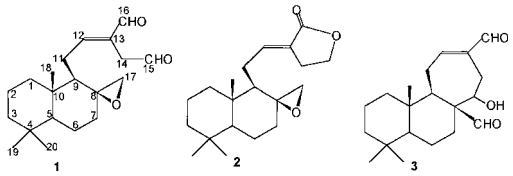
The seeds of *Aframomum daniellii* (Hook f.) K. Schum (Zingiberaceae) known in the Western Province of Cameroon as "Achoh" are the most medicinally used part of this species and are believed to be eaten by snakes to facilitate sloughing. A hot-tasting diterpene, (*E*)-8β(17)-epoxylabd-12-ene-15,16-dial (aframodial, **1**) first isolated from this species in our laboratory in 1979 [1] has since been obtained from other sources albeit in relatively smaller yields [2, 3]. Aframodial displays a wide spectrum of biological activities [2–5]. Of particular significance is its antifungal activity [4]. Aframodial also exhibits strong cytotoxic activity towards KB cells [2] and towards L1210 (ED_{50} 2.5 μg/ml) cells [4]. Tanabe et al. [3] have also shown the antihypercholesteromic effect of aframodial which resulted from its inhibitory activity on hydroxyglutaryl-coenzyme A (HMG-CoA) reductase.



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As a continuation of our programme aimed at the isolation of naturally occuring compounds with potential anticancer, antiviral and antiprotozoal activities, we have submitted aframodial to the NCI anticancer screening programme. We now report its *in vitro* antileukemic activity as well as the identification of two minor antifungal diterpenoids [2], galanolactone (**2**) and galanal B (**3**) not previously isolated from any Aframomum species.

In contrast to previous results from our laboratory [4–6], aframodial (**1**) was isolated in a lower yield from the hexane extract of the fine powdered seeds of *Aframomum daniellii* together with two other minor components **2** and **3**. Aframodial, with a 50 mg/kg toxicity index, showed reproducible activity in the NCI *in vitro* human cell line screen [7] against the whole leukemia cell line panel (CCRF-CEM, HL-60, K-562, MOLT-4, RPMI-8226, SR lines), and the NCI-H522 non-small cell cancer lung (NSCLC) line at concentrations of 10^{-4} to 10^{-5} molar. It was advanced into the NCI in vivo hollow fiber assay [8] where, on intraperitoneal (IP) administration, it exhibited cell kill against NCI-H522 cells contained in IP implanted cancer lung (NSCLC) and OVCAR-3 ovarian cells contained in fibers implanted subcutaneously (SC). On the basis of the cell kill observed against the NCI-H522 cell lines in this assay, aframodial was approved for testing against a range of human tumor xenograft systems. However, no significant activity was observed against SC implanted NCI-H522 and NCI-H23 tumors on IP administration of the drug on an intermittent schedule (QDx5 and Q4Dx3, respectively, both starting on day 16) at doses ranging from 2.7 to 10 mg/kg nor against the SC implanted OVCAR-3 tumor on intermittent IP (Q4Dx3 starting on day 7) and IV (QDx5 and Q4Dx3, both starting on day 8) administration at doses from 2.7 to 18 mg/kg. In view of the lack of a significant *in vivo* activity against