



Pharmacological properties of the extract and some isolated compounds of *Clausena lansium* stem bark: Anti-trichomonal, antidiabetic, anti-inflammatory, hepatoprotective and antioxidant effects

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ABSTRACT

Ethnopharmacological Relevance: *Clausena lansium* (Fool's Curry Leaf) is used for various ethnomedical conditions in some countries, including bronchitis, malaria, viral hepatitis, acute and chronic gastrointestinal inflammation, and as a spicy substitute of the popular Curry leaf tree (*Murraya koenigii*).

Aim of the study: This study was to evaluate the ethnomedical uses of the stem bark in inflammatory conditions, hepatotoxicity and to determine the anti-diabetic and anti-trichomonal properties of the plant.

Materials and Method: Anti-trichomonal, *in vivo* and *in vitro* antidiabetic and insulin stimulating, anti-inflammatory, hepatoprotective and anti-oxidant activities using *Trichomonas gallinae*, glucose loaded rats and *in vitro* insulin secreting cell line (INS-1 cell), carrageenin-induced rat paw oedema, CCl₄-induced hepatotoxicity and DPPH scavenging ability methods respectively for the extracts and some isolates were determined.

Results: A dichloromethane extract was superior over methanolic extract with respect to an anti-trichomonal activity which was measured after 24 and 48 h. The isolated compounds imperatorin and 3-formylcarbazole had the main anti-trichomonal activity (LC_{50s} of 6.0, 3.0 and 3.6, 9.7 µg/mL after 24 and 48 h, respectively). Methanolic extract (100 mg/kg) induced maximum and significant ($p < 0.05$) anti-hyperglycaemic activity of 15.8% at 30 min and a 38.5% increase in plasma insulin at 60 min, compared to control. The increase in plasma insulin after 60 min, compared to 0 min, was 62.0% ($p < 0.05$). The significant 174.6% increase of insulin release from INS-1 cells (*in vitro*) at 0.1 mg/ml indicates that it mediates its antidiabetic action mainly by stimulating insulin release. Imperatorin and chalepin were the major active constituents increasing *in vitro* insulin release to 170.3 and 137.9%, respectively. 100 mg/kg of the methanolic extract produced an anti-inflammatory activity after 4 h. A sedative effect was not observed. 100 and 200 mg/kg of methanolic extract administered *i.p.*, reduced CCl₄-induced hepatotoxicity firstly by 5.3 and 8.4% reduction in phenobarbitone-sleeping time respectively, secondly by reversing the reduction in serum liver proteins by 7.0–8.8%, serum AST, ALT and ALP activities by 27.7–107.9% and thirdly by diminishing increased values of plasma AST, ALT and ALP activities by 13.2–83.8%. The extract exhibited antioxidant activities.

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Conclusion: The hepatoprotective activity of *C. lansium* is partly due to its anti-oxidant and anti-inflammatory properties and confirms its folkloric use in the treatment of gastro-intestinal inflammation, bronchitis and hepatitis. In addition the use of *C. lansium* stem bark would be useful in diabetes and trichomoniasis.

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1. Introduction

Clausena lansium (Lour.) Skeels (syn. *Clausena wampi* (Blanco) Oliv.; *Clausena punctata* (Sonn.) Rehd. & Wils.; *Cookia punctata* Sonn.; *Cookia wampi* Blanco; *Quinaria lansium* Lour.) is a minor member of the Rutaceae. It is an attractive shrub or small tree with somewhat grapelike fruits similar to the citrus fruits and commonly called Wampee, False or Fool's Curry (Fletcher, 2001; Zhao et al., 2004). In Taiwan and China, the leaves have been used as a folk medicine for the treatment of coughs, asthma and gastro-intestinal diseases and the seeds for gastro-intestinal diseases such as acute and chronic gastro-intestinal inflammation, ulcers, etc. The fruit has stomachic and cooling effects and is used ethnomedically as a vermifuge and digestive disorders (Lin, 1989). The halved, sun dried immature fruits and slices of dried roots and stems are used in Vietnam and oriental remedies for bronchitis and malaria (Li et al., 1991; Kumar et al., 1995; Zhao et al., 2004). Different parts are used in the treatment of acute and chronic viral hepatitis in Chinese local medicines (Yang et al., 1988; Liu et al., 1996; Fletcher, 2001).

The C₁₃- and C₁₈-carbazole alkaloids, simple and prenylated coumarins, furocoumarins, amines and novel amides were isolated from different parts of the plant, indicating similarities in their constituents (Khan et al., 1983; Yang et al., 1988; Lakshmi et al., 1989; Lin, 1989; Li and McChesney, 1990; Li et al., 1991; Kumar et al., 1995; Liu et al., 1996; Adebajo et al., 1998; Ito et al., 1998; Wu and Li, 1999; Zhao et al., 2004). Some pharmacological effects such as anti-lipid peroxidation, cerebral protective, hepatoprotective, hypoglycaemic, anticonvulsant, cardiovascular and antitumour have been reported for some coumarins and amides, such as clausenamamide and clausenacoumarin, isolated from the leaf (Shen et al., 1989; Li et al., 1991; Liu et al., 1991, 1996). However, the stem bark has not been investigated in detail.

Trichomonas vaginalis and *Trichomonas gallinae* cause trichomoniasis in man and animals with terrible medical implications; and metronidazole is the drug of choice. Increasing resistance of micro-organisms and parasites to the standard drugs and high cost of orthodox medicine that keeps it out of the reach of common man, especially the rural dwellers are problems. Hence, the current velocity of investigations into the available wealth of ethnomedicinal or folkloric usage of medicinal plants and other natural products must be maintained to achieve the WHO goal of "health for all". Since *C. lansium* is used as a substitute for Curry Leaf, we were interested to see whether it also has similar anti-trichomonal and anti-diabetic activities recently reported for *Murraya koenigii* (Adebajo et al., 2004, 2006; Kesari et al., 2005). Furthermore, carbon tetrachloride (CCl₄) is a common poison (model in toxicology) that induces damage to the liver and other tissues by a mechanism believed to involve membrane lipid peroxidation. Its toxicity on liver tissues has been shown to be due to formation of free radicals and produced inflammatory responses (Reckanagel and Glende, 1973). Relationship has been given between liver inflammation and hepatotoxicity using SGPT (Serum-Glutamat-Pyruvat-Transaminase) and hepatoprotective constituents of *C. lansium* leaf (Liu et al., 1996). Since the ethnomedical uses of the plant for coughs, acute and chronic gastro-intestinal inflammation (Lin, 1989), bronchitis (Li et al., 1991; Kumar et al., 1995; Zhao et al., 2004), acute and chronic viral hepatitis (Yang et al., 1988; Liu et al., 1996) are all related to inflammation, and since a relationship exists between inflammation and hepatotoxicity,

the anti-CCl₄ hepatoprotective role of the methanolic extract of *C. lansium* stem bark was evaluated by determining the modification of the phenobarbitone-induced sleeping time (PIST) and the activity of some liver enzymes. Therefore, this study is focussed on the evaluation of the ethnomedical uses of *C. lansium* stem bark in inflammatory conditions, hepatotoxicity and to determine the usefulness of the plant part in diabetes and trichomoniasis.

2. Materials and methods

2.1. Chemicals

Metronidazole (Aventis Pharma, Germany); Heparin-Natrium-25,000 (Ratiopharm, GmbH, Ulm, Germany) and Glibenclamide was from Sigma–Aldrich, 3050 Spruce St, St. Louis, MO 63103, USA. Halothane was from Fluka Chemie GmbH, Steinheim, Germany. Rat insulin was from Novo Nordisk, Bagsvaerd, Denmark; (mono-¹²⁵I-Tyr A¹⁴)-porcine insulin was from Sanofi-Aventis, Frankfurt, Germany, and anti-insulin antibodies were from Linco, St. Louis, USA. Carbon tetrachloride (Merck, Germany); Phenobarbitone (May and Baker, Dagenham); Randox *in vitro* Diagnostic medical kits (AL 100–AST, AP 501–ALT); Carrageenin and DPPH-1,1-diphenyl-2-picryl-hydrazyl (Sigma); Methanol (BDH) and Olive oil was from Goya en Espana, S.A. Seville, Spain.

2.2. INS-1 cells (rat insulinoma cell line, insulin secreting cell line)

Dr. C.B. Wollheim, Geneva, Switzerland generously provided INS-1 cells, an insulin releasing insulinoma cell line. Biological chemicals/media used for the INS-1 cell experiment were obtained as previously reported (Adebajo et al., 2007).

2.3. Animals

Healthy Wistar rats (200–260 g) of either sex bred under standard conditions (temp. 27 ± 3 °C, relative humidity 65%) at the animal house, Department of Pharmacology, Faculty of Pharmacy, O.A.U., Ile-Ife, Nigeria and Wistar albino rats of either sex (240–600 g) maintained at 22 °C under natural 12 h daylight/night conditions in the Animal House of the Institute of Pharmaceutical and Medicinal Chemistry, Münster, Germany, were kept for at least 5 days before commencement of the experiment. They were fed on a standard pellet diet (Bendel Feeds, Nigeria or Altromin, Lage, Germany) and water was given *ad libitum*. The "principle of laboratory animal care" (NIH publication No. 85-23) guidelines and procedures were followed in this study (NIH publication revised, 1985).

2.4. Groups of rats

Five groups of 5–8 normal rats each were fasted for 18 h and variously treated with either extract, adequate positive controls or only 0.9% (w/v) saline (0.5 mL, vehicle, negative control). Rats were sedated with Halothane before administration of saline/extract/drug as necessary.

2.5. Culture of INS-1 cells and insulin radioimmunoassay

The INS-1 cells were grown in 24 multi-wells for 5–6 days (half confluence: 1–2 × 10⁶ cells/mL) in RPMI (Roswell Park Memorial Institute) medium supplemented with 10% (v/v) fetal calf

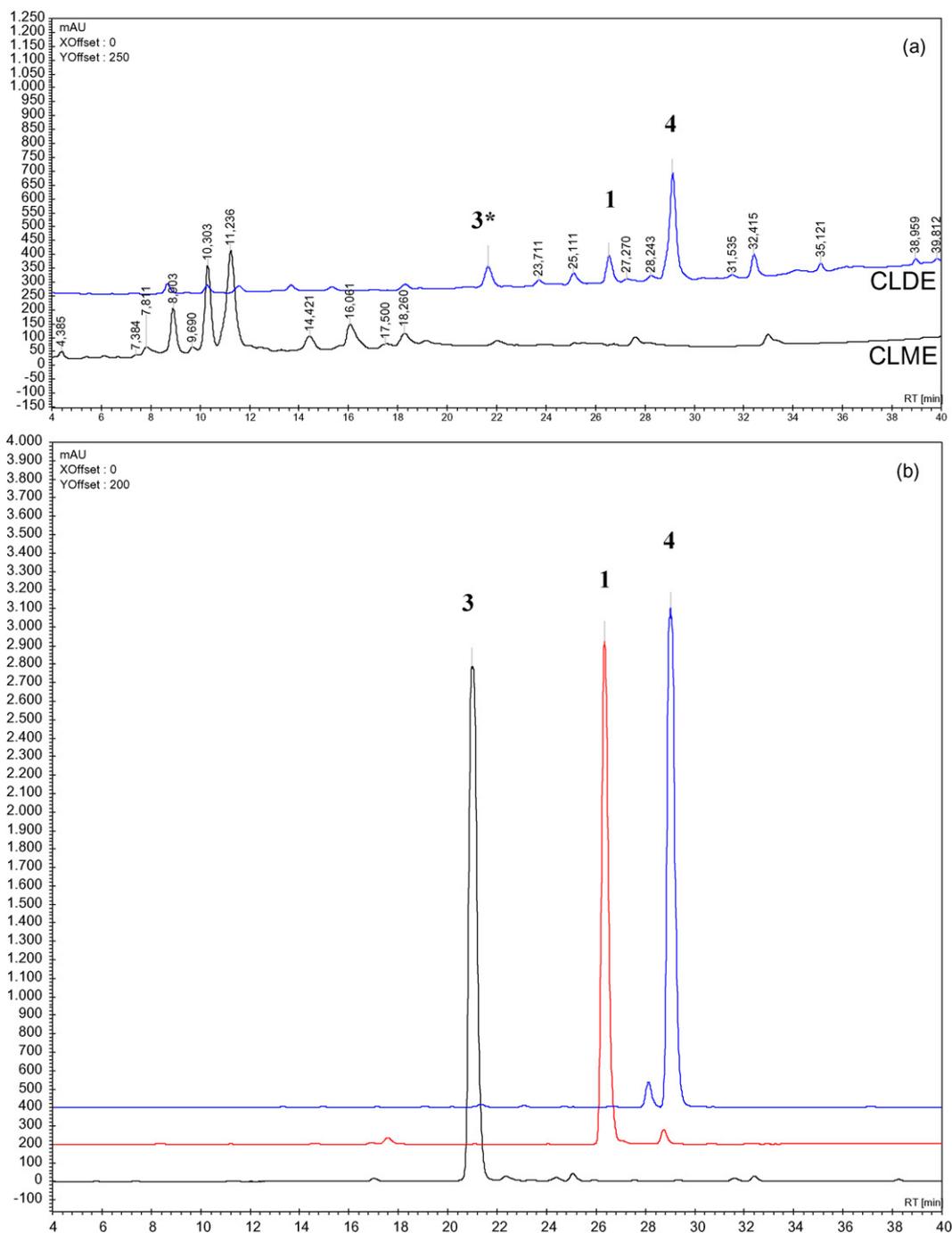


Fig. 1. HPLC analysis of *C. lansium* stem bark extracts. (a) HPLC profiles of the dichloromethane and methanolic extracts recorded at 215 nm. *The peak of 3-formylcarbazole (3) showed a slightly divergent UV spectrum indicating that it co-elutes with an unknown constituent. (b) HPLC chromatograms of imperatorin (1), 3-formylcarbazole (3) and chalepin (4) recorded at their respective absorption maxima (300, 328, 336 nm, respectively). (For HPLC conditions and quantification results see Section 2.7.)

serum, 100 U/mL penicillin and 0.1 mg/mL streptomycin. Prior to the experiment, INS-1 cells in multi-wells were washed 3 times and incubated with Krebs–Ringer buffer containing 10 mmol/L HEPES (N-(2-hydroxyethyl)piperazine-N'-2-ethane-sulphonic acid) and 0.5% bovine serum albumin (KRBH) and 5.6 mM glucose for 90 min. Insulin released into the medium was determined by a radioimmunoassay using rat insulin as standard, (mono-¹²⁵I-Tyr A¹⁴)-porcine insulin as the labelled compound and anti-insulin antibodies. Each extract/compound had been checked for non-interference with the insulin radioimmunoassay (Adebajo et al., 2007). The value of insulin release induced by 5.6 mmol/L glucose was taken as 100% (negative control) and the results of other test

agents were expressed as percentage of this value. Data were not given as absolute amounts of secreted insulin because cultured cells did not grow identically from week to week at a distinct time point after the passage. The extracts, the pure isolates and glibenclamide (positive control) were tested together with 5.6 mM glucose. The insulin secretion stimulated by 3.0 mmol/L glucose was taken as a sub-stimulatory concentration (control experiment).

2.6. Plant material, extraction and isolation

The authentication and collection of *C. lansium* stem bark was done as previously reported (Adebajo et al., 1998). The succes-

sive extraction of dried stem bark with dichloromethane (CH₂Cl₂ 12.7 g) and methanol (MeOH 27.0 g), isolation of imperatorin (**1**), phellopterin (**2**), 3-formylcarbazole (**3**) and chalepin (**4**) from the dichloromethane extract were as described (Kumar et al., 1995; Adebajo et al., 1998).

2.7. HPLC-profiles of the dichloromethane and methanolic extracts and peak assignment as well as approximate quantification of isolates **1** and **4**

The peaks of compounds **1**, **3** and **4** in the HPLC chromatogram of the dichloromethane extract were readily identified in comparison with the isolated compounds used as reference samples. HPLC was performed on a Nucleodur column, 100-C₁₈, 5 μm, dimension: 125 mm × 4.6 mm (Macherey-Nagel, Düren, Germany) eluted with gradient mixtures of MeOH–H₂O (30% MeOH at 0 min; 70% MeOH at 30 min; 100% MeOH at 45 min held up to 55 min; oven temperature: 25 °C. Flow rate: 1.4 mL/min. Detection: UV-DAD MD 2010 (Jasco, Groß-Umstadt, Germany) 200–450 nm.; injection volume: 10 μL; sample concentration: 10 mg/mL. Chromatograms of dichloromethane and methanol depicted in Fig. 1a were recorded at 215 nm. The approximate concentration of compounds **1** and **4** in dichloromethane was assessed by integration of chromatograms recorded at the respective UV absorption maxima at 300 and 336 nm (shown for the reference compounds in Fig. 1b). The peak areas of the reference compounds **1** and **4** corresponded to 28.4 and 27.0 (mAu min)/μg, respectively and determined in the dichloromethane sample (10 mg/mL, 10 μL injected) were 16.3 and 181.5 mAu min, corresponding to 0.57 and 6.7 μg/100 μg extract, respectively. The concentration of compound **3** was not determined due to insufficient peak purity. However, it can be expected from the size of the peak that the total content of compound **3** in the extract is below 1%.

2.8. Anti-trichomonal activity

Trichomonas gallinae isolated from a pigeon was mixed with normal saline, then distributed into test tubes of Ringer's egg-serum culture for enteric protozoan and incubated at 37 °C for growth. Serial dilutions made from Stock solutions of dichloromethane and methanolic extracts, the four isolates **1**, **2**, **3**, and **4** from dichloromethane and metronidazole (dissolved in 1 mL DMSO) were incubated in a steam incubator at 37 °C for 24 and 48 h and tested using serial dilutions. The number of organisms per millilitre in each well for 0, 24 and 48 h were counted using the microscope and LC₅₀ and LC₉₀ values calculated for short and long exposure periods (Adebajo et al., 2006).

2.9. Anti-diabetic study

2.9.1. Glucose loaded hyperglycaemic rats

A glucose tolerance test was performed by giving 0.6 mL glucose solution (10 g/kg, p.o.) to rats. Rats with blood glucose level > 7.0 mmol/L (126 mg/dL) after 1 h were presumed hyperglycaemic and used for further experiments. 100 mg/kg of methanolic extract was given 1 h after the glucose load (time point 0). Blood was drawn by the retrobulbar technique using heparinised capillary tubes, and the samples (15 drops) were collected into chilled tubes at time points 0, 30, 60 and 120 min. The blood sampling procedure did not take longer than 20 s. Blood was used immediately for the glucose determination after having added haemolysis reagent (maleimide 0.24 mg, digitonin 0.12 mg and 50 mL water) or kept frozen for a maximum of 10 days. After having added 150 IU heparin-Na to the blood, the centrifuged plasma (4000 rpm for

10 min, 10 °C) was kept frozen at –20 °C until the insulin radioimmunoassay was performed.

2.9.2. Blood glucose determination

Blood glucose was determined by the glucose oxidase method (Glucoquant®, Roche Diagnostics GmbH, Mannheim, Germany). Under assay conditions, glucose calibration curves were linear over the range 0–200 mg/dL. The absorbance of the enzymatic products was determined at 334 nm using a photometer UV-160A, (Shimadzu, Germany). The measurement of the samples (duplicates) was performed after 10 min.

2.9.3. Insulin determination

Either 100 μL plasma (*in vivo* experiments) or 2.5 μL of supernatant of incubated INS-1 cells (*in vitro* experiments) were assayed with a radioimmunoassay using rat insulin as a standard, (mono-¹²⁵I-Tyr^{A14})-porcine insulin as the labelled compound and anti-insulin antibodies. For calibration, rat insulin between 0.39 and 25 μU/100 μL was used. There was no direct interference of the tested compounds with the radioimmunoassay. For *in vitro* experiments concentrations of 0.01 and 0.1 mg/mL of extract/isolate or 0.001 mg/mL glibenclamide (control) was used.

2.10. Hepatoprotective effect, phenobarbitone-induced sleeping time, protein and enzymes determinations

100 and 200 mg/kg of the methanolic extract and 5 mL/kg of liver tonic (positive control) and 1 mL/kg of saline and olive oil (vehicle, negative control) were given at 48, 24 and 2 h before an i.p. administration of 1.5 mL/kg dose of 50% (v/v) of CCl₄ in olive oil to induce hepatic injury. Phenobarbitone (40 mg/kg, i.p.) was given 4 h after CCl₄ administration to the rats and the sleeping time (rats without reflex) was recorded in each group.

Blood was collected from diethyl ether anaesthetized rats by cardiac puncture using sterile disposable syringe containing anticoagulant 3.8% Trisodium citrate and plasma was obtained by centrifugation (6000 rpm for 10 min) for subsequent determinations. The livers were rapidly excised, weighed and rinsed with ice-cold sucrose (0.25 M) solution in an ice-bath, then 1 g was homogenized in 10 mL of ice-cold 0.25 M sucrose solution using a motor driven glass-teflon Potter-Elvehjem (TRI-R STIR-S model K₄₃) and passed through 2 layers of pre-cooled gauze to remove particulate matter before performing the assay.

The protein concentrations of the plasma and the liver homogenate were determined by the method of Lowry et al. (1951) using bovine serum albumin as a standard. Aspartate aminotransferase (AST), alanine aminotransferase (ALT) in the plasma and liver were determined by a slight modification of the Reitman and Frankel's method (Adebajo et al., 2006) using a commercially available Randox kit (Randox Laboratories Ltd., U.K.). Alkaline phosphatase (ALP) was determined according to the optimized standard method of the Deutsche Gesellschaft für Klinische Chemie (DGKC).

2.11. Sedative effect of the extract

In a separate experiment, fresh groups of rats given 100 and 200 mg/kg of methanolic extract, phenobarbitone (40 mg/kg) and saline, respectively were observed over a period of ten (10) hours for loss of righting reflex, an index of sleep.

Table 1
Anti-trichomonal activities of extracts and various isolates. Metronidazole (dissolved in DMSO) is used as a positive control. LC₅₀ and LC₉₀: concentrations at which 50 and 90% parasites of *Trichomonas gallinae* were killed after indicated time-points; N = 6.

Extracts/isolates	Inhibition of <i>Trichomonas gallinae</i> at 24 h		Inhibition of <i>Trichomonas gallinae</i> at 48 h	
	LC ₅₀ (µg/mL)	LC ₉₀ (µg/mL)	LC ₅₀ (µg/mL)	LC ₉₀ (µg/mL)
Dichloromethane extract	19.0	242.0	7.8	185.0
Methanolic extract	2.0	460.0	5.0	527.5
Imperatorin (C ₁₆) (1)	6.0	230.0	3.0	35.0
Phellopterin (C ₁₇) (2)	15.2	155.9	60.4	254.8
3-Formylcarbazole (C ₁₃) (3)	3.6	243.9	9.7	41.4
Chalepin (C ₁₉) (4)	22.4	236.2	30.5	257.4
Metronidazole	1.9	3.5	2.0	5.0

2.12. Anti-inflammatory activity

1 h before injection of 0.1 mL of 1% carragenin suspension, 100 and 200 mg/kg of methanolic extract, 10 mg/kg of indomethacin (positive control), mixture of normal saline and olive oil (1 mL/kg each) as vehicle were separately injected i.p. into sub-plantar surface of the right hind limb. The paw volume was measured by plethysmometer (Ugo Basile, 21025 Comerio-Varese, Italy) to determine the oedema formation size after 0, 0.5, 1.0, 2.0, 3.0 and 4.0 h (Winter et al., 1962).

2.13. Antioxidant assay

The free radical scavenging activity was analysed using the DPPH assay (Sanchez-Moreno et al., 1998). The reaction mixture consisted of 1 ml of 100 mM acetate buffer (pH 5.5), 1 ml of 100% ethanol and 0.5 ml of 2.5 mM ethanol (100%) solution of DPPH. A 0.5 ml of the test substances in the appropriate concentration was then added to make 0.2, 0.5 and 1.0 mg/ml of methanolic extract and 0.01 mg/ml of ascorbic acid. The time-course of the change in absorbance at 517 nm was monitored over 20 min until the reaction plateau step was reached. The percentage of DPPH remaining was calculated according to the equation:

$$\%DPPH_{REM} = \frac{[DPPH]_{(t)}}{[DPPH]_{(0)}} \times 100$$

where [DPPH]₍₀₎ is the remaining concentration of the stable radical without the antioxidant and [DPPH]_(t) is the remaining concentration at the reaction plateau step.

Therefore, % antioxidant activity [AA] = 100 – % DPPH_{REM}.

2.14. Statistical analysis

Analysis of variance (ANOVA) was used, followed by Dunnett range post hoc comparisons to determine the source of significant differences for all determinations except when *in vitro* insulin release, anti-inflammatory, PIST and antioxidant activities were determined and Newman Keul's test was used as the post hoc test. Values of *p* < 0.05 were considered to be of statistical significance.

3. Results

3.1. Identification of the four isolates in the dichloromethane and methanolic extracts by HPLC

There was no major overlap in the HPLC profiles of dichloromethane (CH₂Cl₂) and methanolic (MeOH) extracts (Fig. 1a). While the methanolic extract contained mainly more polar constituents, imperatorin (**1**) and chalepin (**4**) could be

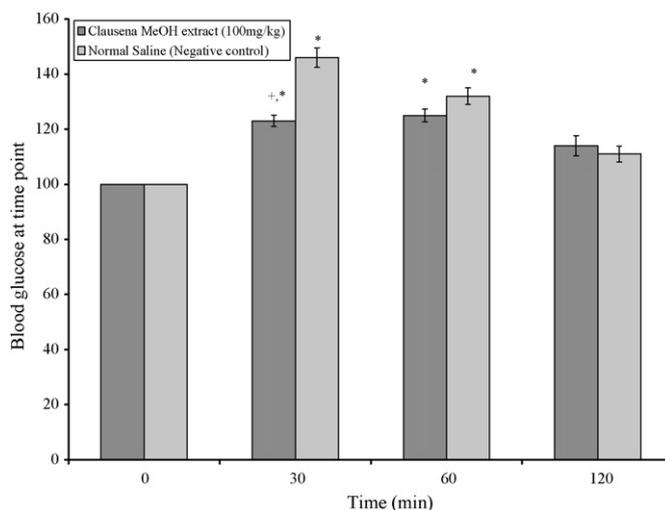


Fig. 2. Time course of blood glucose in glucose loaded rats induced by 100 mg/kg methanolic extract. Means and \pm S.E. are given for N = 6–8 rats. **p* < 0.05 comparison to time-point 0; +*p* < 0.05 comparison with saline (negative control) at indicated time-points determined using ANOVA followed by Dunnett post hoc test.

unambiguously identified in the dichloromethane extract. A peak corresponding to 3-formylcarbazole (**3**) appeared also in the dichloromethane extract, which, however, showed a somewhat different UV spectrum from the reference sample, probably caused by co-elution with an unidentified constituent (Fig. 1b). Phellopterin (**2**) could not be identified unambiguously in the investigated extract samples. Compounds **1** and **4** constituted 0.6 and 6.7%, respectively, of the dichloromethane extract as determined by quantitative analysis based on their absorption maxima at 300 and 336 nm, using the pure compounds as standards.

3.2. Anti-trichomonal activity

The anti-trichomonal activities of the dichloromethane and methanolic extracts, isolated compounds **1**, **2**, **3** and **4** and metronidazole (control) are as shown in Table 1. The LC₅₀ and LC₉₀ after 24 and 48 h treatment are shown. Using LC₉₀ values, dichloromethane extract was more effective than MeOH extract; 3-formylcarbazole and imperatorin were the most effective of the isolated compounds though not fully achieving the effect of metronidazole (positive control).

3.3. Blood glucose reduction

Blood glucose levels of the glucose loaded rats given 100 mg/kg of methanolic extract (test) or saline (control) increased after 0.5 h to the maximum of 123 and 146%, respectively (Fig. 2). The 15.8% lower glucose level of the methanolic extract compared to control was significant at 0.5 h.

Table 2Effects of the extracts and isolates of *C. lansium* stem bark on glucose-mediated insulin release from INS-1 cells.

Compounds	Concentration (mg/mL)	Insulin release (% of glucose effect at 5.6 mM)
Glucose [3.0 mM] (sub-stimulatory concentration control)		62.76 ± 5.89 ^a
Glucose [5.6 mM] (negative control)		100.0
Glucose [5.6 mM] + dichloromethane extract	0.01	73.12 ± 6.53 ^a
	0.1	100.7 ± 11.49
Glucose [5.6 mM] + methanolic extract	0.01	96.85 ± 11.34
	0.1	174.6 ± 8.56 ^c
Glucose [5.6 mM] + Imperatorin (1)	0.01	122.9 ± 7.42
	0.1	170.3 ± 8.85 ^c
Glucose [5.6 mM] + phellopterin (2)	0.01	103.4 ± 5.90
	0.1	71.55 ± 4.92 ^a
Glucose [5.6 mM] + 3-formylcarbazole (3)	0.01	78.00 ± 8.45
	0.1	61.40 ± 4.56 ^a
Glucose [5.6 mM] + chalepin (4)	0.01	96.48 ± 6.99
	0.1	137.9 ± 5.87 ^c
Glucose [5.6 mM] + glibenclamide (positive control)	0.001	173.2 ± 6.45 ^c

INS-1 cells in multi-wells were washed three times and incubated in KRHB-buffer for 90 min at 5.6 mM glucose. The insulin released by 5.6 mM glucose was taken as 100% and the results of others were expressed as percentage of this value. Each value represents the mean ± S.E.M.

^a $p < 0.05$ compared with the effect of 5.6 mM glucose (negative control) and glibenclamide (positive control) was determined by one-way analysis of variance (ANOVA) followed by the Newman Keul's test as the post hoc test. $N = 6-8$.

3.4. *In vivo* and *in vitro* insulin releasing ability

Using the same animal experiments plasma insulin was determined. The extract significantly increased plasma insulin levels after 1 h by 62% (Fig. 3). At this time point the effect was 38.5% higher than that of saline (control) (Fig. 3). The effects vanished after 2 h. Methanolic extract, compounds **1** and **4** dose dependently stimulated insulin release *in vitro* from INS-1 cells while dichloromethane extract, **2** and **3** induced a dose dependent inhibition (Table 2). The values of 174.6, 170.3 and 137.9% for methanolic extract, **1** and **4** at 0.1 mg/mL were close to that of 0.001 mg/mL glibenclamide (positive control) (Table 2).

3.5. Hepatoprotection

3.5.1. Phenobarbitone-induced sleeping time (PIST)

CCl₄ due to its hepatotoxicity caused a 99.7% increase in sleeping time in rats given i.p. 40 mg/kg phenobarbitone. The sleep duration in these rats was dose dependently decreased by 5.3 and 8.4% by the pretreatment with 100 or 200 mg/kg of methanolic extract

respectively while 5 mL/kg liver tonic (positive control) produced a decrease of 76.2% (Fig. 4).

3.5.2. Plasma and liver enzyme activity

The reversal effect of the methanolic extract on CCl₄ induced change in plasma total protein and liver enzyme activities was investigated next. 100 and 200 mg/kg of this extract and Liver tonic (5 mL/kg) reverted decreased and increased AST, ALT and ALP in liver and plasma homogenates respectively as shown in Figs. 5 and 6. The high dose of the extract (200 mg/kg) even reduced the plasma ALP values back to normal (Fig. 6).

3.6. Anti-inflammatory effect *in vivo*

100 and 200 mg/kg of methanolic extract or 10 mg/kg indomethacin in contrast to saline (control) inhibited oedema formation at the 4th hour by 55.5, 27.1 and 64.0%, respectively (Fig. 7). Even better values (same rank order) were obtained after 1 h (Fig. 7).

3.7. Antioxidant activity *in vitro*

Low concentrations (0.2 and 0.5 mg/mL) but not high concentrations of the methanolic extract increased this activity to 47.0 up to 96.4% at 0.5–2 which was comparable with the 62.3–117.8% increases by 0.01 mg/mL ascorbic acid, a standard agent (Fig. 8).

3.8. Sedative effect

100 and 200 mg/kg of the methanolic extract induced a slight sedative effect for 10.3 and 9.4 min respectively while the rats that received only phenobarbitone (40 mg/kg) or saline (control) slept for 117.2 and 0.0 min, respectively (Fig. 9).

4. Discussion

The present study reports for the first time the anti-trichomonal, anti-diabetic, anti-inflammatory, anti-hepatotoxicity and antioxidant activities of *C. lansium* stem bark extracts, albeit some hints on antidiabetic and hepatoprotective activities have been reported for some constituents of its leaf (Shen et al., 1989; Liu et al., 1996). The differences in the anti-trichomonal effects of the two extracts investigated, a methanolic and a dichloromethane extract, were supported by quantitative differences in their compositions as shown by HPLC (Fig. 1a and b). A methanolic extract (or an alcoholic

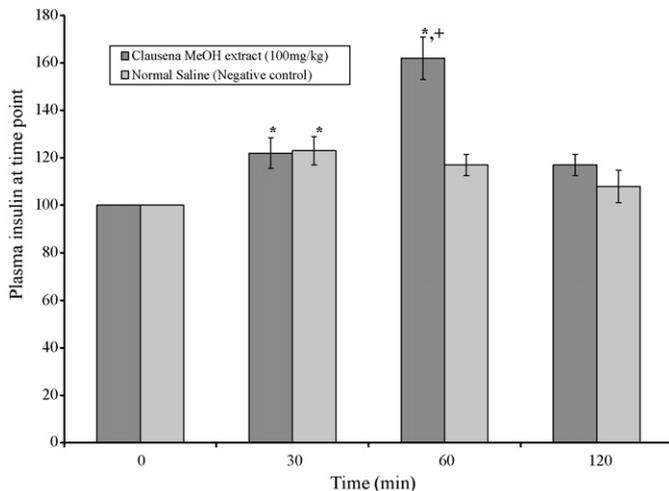


Fig. 3. Time course of plasma insulin releasing abilities in glucose loaded rats induced by methanolic extract (100 mg/kg). Means ± S.E. are shown for $N = 6-8$ rats. ^{*} $p < 0.05$ comparison to time-point 0; ⁺ $p < 0.05$ comparison with saline (negative control) at indicated time-points determined using ANOVA followed by Dunnett post hoc test.

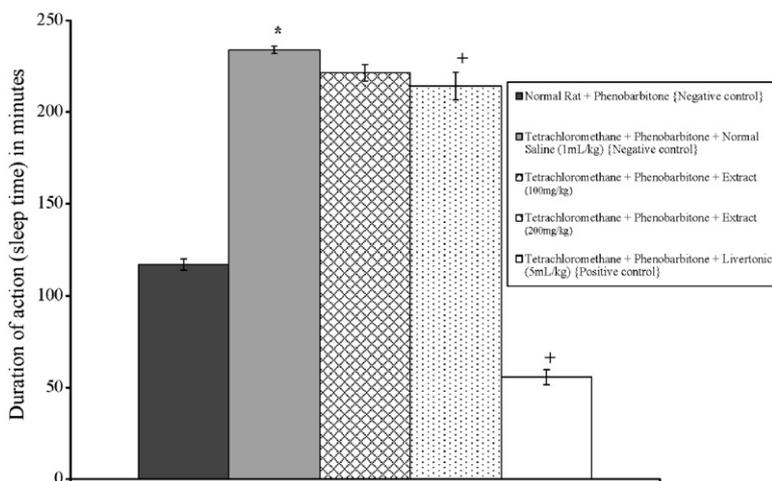


Fig. 4. Effect of the methanolic extract on tetrachloromethane (CCl_4)-induced prolongation of phenobarbitone sleeping time in rats. Values are expressed as means \pm S.E.M. $N=5$. * $p < 0.05$ for CCl_4 -induced hepatotoxic rats compared with those of the normal rats; + $p < 0.05$ for effects of extracts/drug compared with no addition.

ethnomedicinal preparation that would normally be used) of the stem bark that would contain the constituents of both the extracts may be most useful in trichomoniasis.

Since the dichloromethane extract was more active than the methanolic extract (LD_{50} values), the isolates of this extract were further examined for their anti-trichomonal activities. Imperatorin (**1**) and 3-formylcarbazole (**3**) have better activities than the original extract while the poor anti-trichomonal activity of chalepin (**4**), the major compound detected by HPLC, may have contributed to the reduced activity of this extract (Table 1). The presence of a 5-methoxy group in **2** is obviously responsible for the reduction in its activity when compared with that of **1**. Chalepin with higher LD_{50} values than **1** may suggest the importance of the 2',3' double bond in the furano ring and free C-3 to the activity of the coumarins of this plant (Table 1). **1**, **3** and possibly other constituents in the methanol may be the active and synergistically acting compounds of the False Curry, *C. lansium*. Recently carbazole alkaloids that were not acting in synergism were reported as the active constituents of the true Curry Leaf, *Murraya koenigii* (Adebajo et al., 2004, 2006).

The methanolic extract reduced blood glucose by 16% after 30 min (Fig. 2) and stimulated plasma insulin release by 39% after 60 min (Fig. 3) using a glucose tolerance test. The induction of insulin release *in vitro* (to 175%) at 0.1 mg/mL by methanolic extract, confirmed that its hypoglycaemic activity is primarily due to the stimulation of insulin release though extrapancreatic effects cannot be excluded. Imperatorin and **4** induced 170 and 138% insulin secretory response *in vitro* at 0.1 mg/mL that were better than the 101% effect of the dichloromethane extract (Table 2). Since they were shown by HPLC (Fig. 1a and b) to be present in the dichloromethane in significant amounts, especially chalepin (**4**), they can therefore be considered to contribute significantly to the total activity of the extract. The Phellopterin (**2**) inhibited insulin release (Table 2) and similar to the anti-trichomonal activity, a free C-5 may be important for the *in vitro* insulin secretory abilities of the furocoumarins of the False Curry. However, the strong inhibitory activity of 3-formylcarbazole (**3**) becoming evident at 0.1 mg/mL (Table 2), apparently has no pronounced effect on the overall activity of the dichloromethane. This could be due to its low concentration in

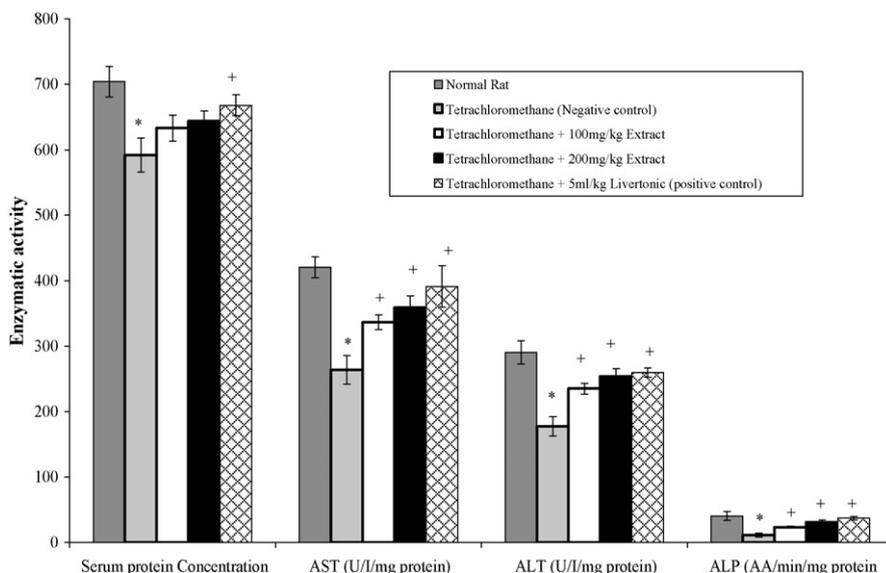


Fig. 5. Effect of the methanolic extract on the concentrations of total serum proteins, on AST, ALT and ALP activities in rat liver after tetrachloromethane (CCl_4) poisoning. * $p < 0.05$ (ANOVA followed by Dunnett's post hoc test) for CCl_4 -induced hepatotoxic rats compared with those of the normal rats; + $p < 0.05$ when values of the CCl_4 -induced rats given extract/drug were compared with those given only normal saline (negative control). Values are expressed as means \pm S.E.M. $N=5$.

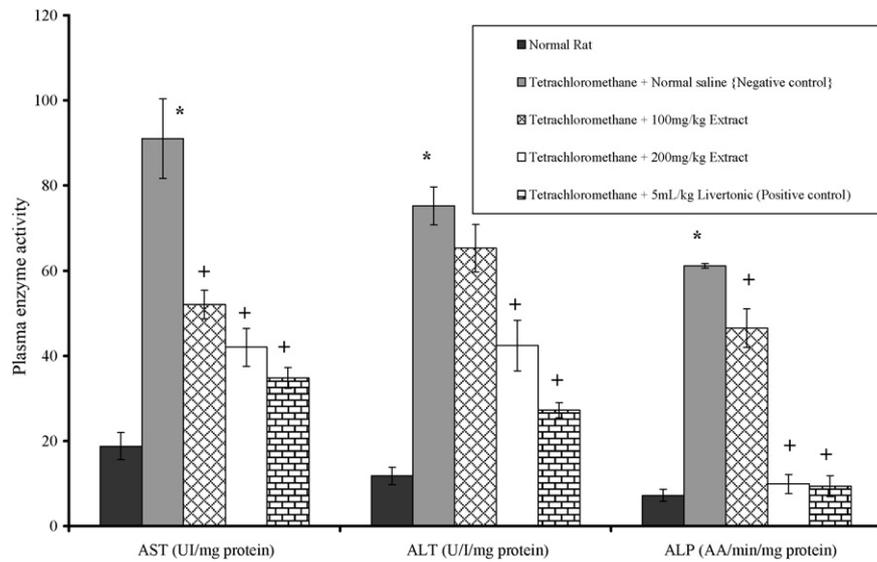


Fig. 6. Effect of the methanolic extract of *C. lansium* stem bark on plasma enzymatic activity in rats with tetrachloromethane (CCl₄)-induced liver damage. * $p < 0.05$ (ANOVA followed by Dunnet's post hoc test) for CCl₄-induced hepatotoxic rats compared with those of the normal rats; + $p < 0.05$ when values of the CCl₄-induced rats given extracts/drug were compared with those given saline only (negative control). There were also significant differences between the enzyme lowering activities of ALT and ALP given by 100 and 200 mg/kg of the extract. Similar enzyme lowering activities for AST and ALP were given by the extract (200 mg/kg) and livertonic (5 mg/kg). Values are expressed as means \pm S.E. $N = 5$.

this extract being 1%. Other unidentified constituents detected in the methanolic extract could be responsible for its interesting *in vivo* increasing insulin levels (Fig. 3). Thus, contrary to the anti-trichomonal activity, the use of the methanolic extract of the False Curry as an anti-diabetic preparation is to be preferred. Hypoglycaemic activity has also been reported for clausenacoumarine, a constituent of the False Curry Leaf (Shen et al., 1989). Therefore, the use of this plant, False Curry, as a substitute for *Murraya koenigii* in spicing foods would give anti-trichomonal and anti-diabetic activities similar to the true Curry Leaf.

Liver cells contain various enzymes and participate in a variety of metabolic activities. Liver damage is caused by excessive consumption of alcohol, viral diseases (hepatitis), obstruction and infections

of the bile ducts, poisons, e.g. CCl₄ (common hepatotoxic compound used in experimental liver toxicity models), etc. The nature of the initiating liver damage with CCl₄ consists of lipid peroxidation resulting in free radical generation. Therefore, inhibition of the generation of free radicals is very important in the protection of the liver against various injuries, e.g. CCl₄-induced injury (Sharma et al., 1989; Liu et al., 1996; Janbaz et al., 2002). There were significant 100% increase in the sleeping time of the phenobarbitone treated (PIST) rats due to CCl₄ intoxication (Fig. 4), decreased AST, ALT and ALP activities of the liver homogenates and serum total protein by 37, 39, 72 and 16% (Fig. 5) with concomitant respective increases of 384, 537 and 749% in the plasma AST, ALT and ALP values respectively, due to CCl₄ intoxication (Fig. 6). Liver damage following toxic

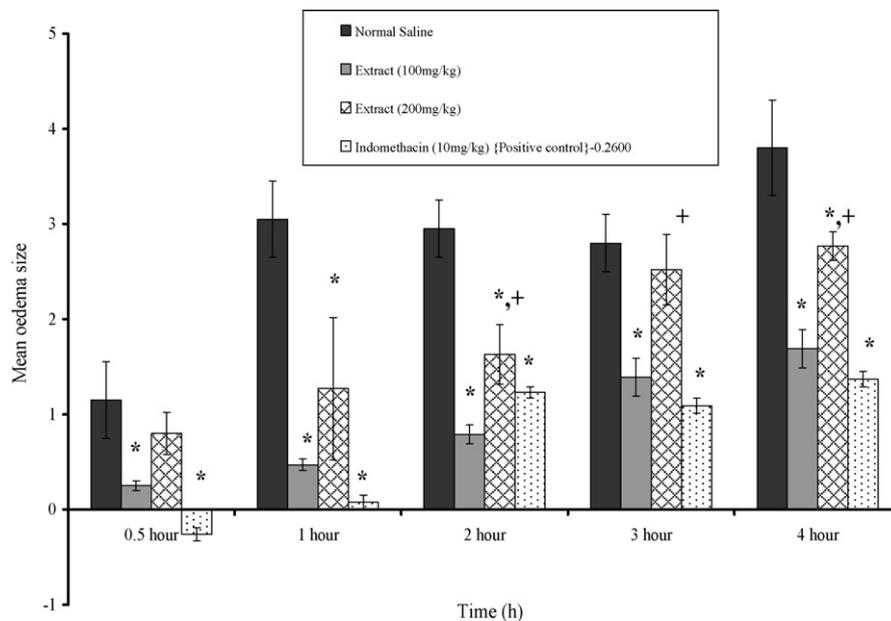


Fig. 7. Time course effect of methanolic extract (100, 200 mg/kg) on carrageenin-induced rat paw oedema. Means \pm S.E. for $N = 5$ rats. * $p < 0.05$ compared with saline (negative control) at the specific time-point; + $p < 0.05$ comparison between corresponding time-point values given 100 and 200 mg/kg of extracts determined using ANOVA followed by Newman-Keul post hoc test. The lower dose was significantly more active in its percentage inhibitions at 2, 3 and 4 h.

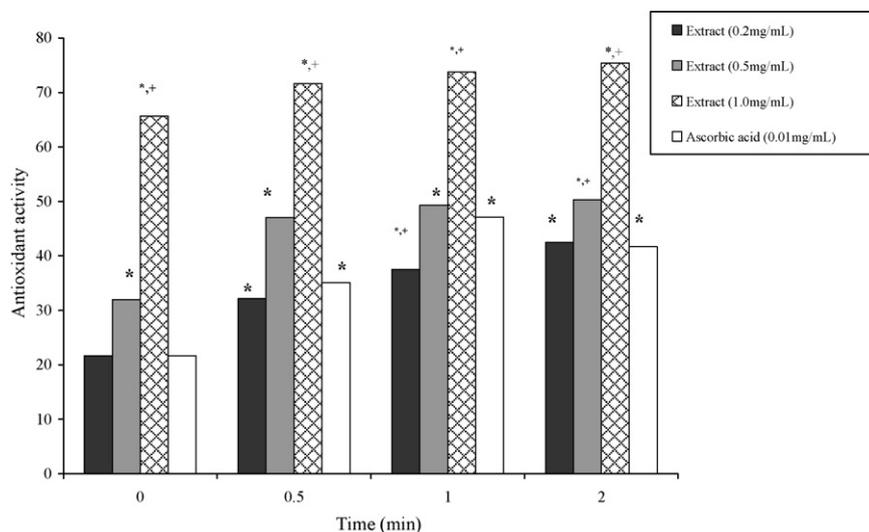


Fig. 8. Antioxidant effect (*in vitro*) of *C. lansium* stem bark extract against 1,1-diphenyl-2-picryl-hydrazyl (DPPH) scavenging abilities. Statistical significance at $*p < 0.05$ by comparing the values of the extract at a particular concentration at specific time-points against those of their respective time T_0 and also with the values of ascorbic acid and other concentrations of the extracts at the corresponding specific time-points using ANOVA followed by Students–Newman–Keul post hoc test.

challenge of chemicals or infectious agents, has often resulted in the rapid release of ALT and AST into the blood circulation and the increases of these two enzymes in the serum are indicative of liver damage (Shen et al., 1989; Janbaz et al., 2002). Similarly, the CCl_4 -induced changes in the plasma and liver activities of these 'liver – marker enzymes' AST, ALT and ALP as well as the decline in the plasma protein level were significantly and dose dependently counteracted by pre-treatment with the methanolic extract (100 and 200 mg/kg) (Figs. 5 and 6). The extent of these reversals given by the extract (200 mg/kg) compared favourably with that of 5 mL/kg of livertonic, a natural standard drug used (Figs. 5 and 6). Thus hepatocellular damage induced by CCl_4 can be overcome *in vivo* by the anti-hepatotoxic activity of the extract.

Using a similar model, hepatoprotective activities have been reported for *Murraya koenigii* leaves (Adebajo et al., 2006). Reversal of elevated serum glutamic pyruvic transaminase (SGPT) by cycloclausenamide, clausenamide coumarin and zeta-clausenamide was used in identifying them as the most potent of nine hepatoprotective constituents isolated from *C. lansium* leaf (Liu et al., 1996). None of these constituents were isolated from the stem bark. Therefore,

this report for the stem bark using other liver enzymes represents the first report of this activity for this plant part, provides an additional proof of hepatoprotective activity for the plant and justifies the use of the stem bark as one of the different parts of the plant used in the treatment of acute and chronic viral hepatitis in Chinese local medicines (Yang et al., 1988; Liu et al., 1996).

Carrageenin is able to induce inflammation of the rat paw reaching its peak activity at 4 h. The highest and most significant anti-inflammatory activity of the extract was already obvious at 1 h after induction of inflammation while the activities of the two used doses stayed significantly different between 2 and 4 h. Even the lower dose (100 mg/kg) with a 56% inhibition of oedema formation after 4 h was more active and comparable with the 64% inhibition induced by indomethacin (10 mg/kg). The 73% inhibition given by this dose was better than the 58% of inhibition produced by indomethacin at 2 h (Fig. 7) and may justify the folkloric use of the plant in acute and chronic gastro-intestinal inflammation and other inflammatory conditions, such as hepatitis and bronchitis (Lin, 1989).

The 9–96% increases in antioxidant activities provided by the methanolic extract were both concentration and time dependent; surprisingly the % increases of their maximal activities decreased with increasing concentration. These activities were comparable with those of 62–118% of ascorbic acid (Fig. 8) indicating that the extract inhibited DPPH radical formation *in vitro* and confirmed its radical scavenging ability. Significant anti-lipid peroxidation, manifested through its oxygen free radical scavenging activity, has already been reported for clausenamide isolated from the leaf of the plant (Liu et al., 1991, 1996). There exists a link of antioxidants with respect to scavenging Reactive Oxygen Species (ROS) and anti-inflammatory effects and therefore play an important role in the treatment of inflammatory diseases (Conner and Grisham, 1996). The demonstration of both antioxidant and anti-inflammatory (carrageenin model) activities by *C. lansium* stem bark extract may confirm this relationship. Hence, these activities may justify the ethnomedicinal use of the plant in gastro-intestinal inflammation (Lin, 1989), bronchitis and hepatitis which are inflammatory conditions occasioned by infection of the lungs and liver, respectively (Liu et al., 1996; Fletcher, 2001). The ROS generated endogenously or exogenously are also associated with the pathogenesis of other diseases such as atherosclerosis, diabetes, cancer, arthritis and aging process (Guyton et al., 1997; Halliwell and Gutteridge, 1999). Hence,

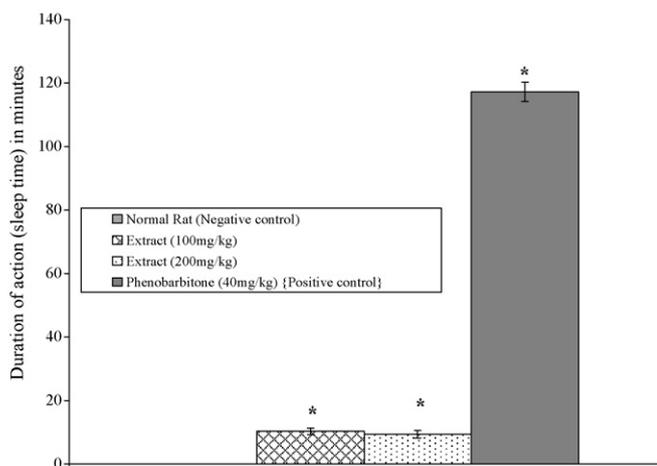


Fig. 9. Sedative effects of the methanolic extract (100, 200 mg/kg) in normal rats. Means \pm S.E. are shown for $N = 5$ rats. $*p < 0.05$ comparison between values of animals treated with extract/drug and those given saline (negative control) determined using ANOVA followed by Dunnett post hoc test.

the demonstrated complementary activities of anti-diabetic, anti-inflammatory, anti-hepatotoxic and anti-oxidant activities of *C. lansium* stem bark extract may all be related and mediated through its anti-inflammatory and anti-oxidant activities.

5. Conclusion

The present study reports for the first time the anti-trichomonal, anti-diabetic, anti-inflammatory, anti-hepatotoxic and anti-oxidant activities of *C. lansium* stem bark extract and justified the ethnomedicinal use of the plant in gastro-intestinal inflammation, bronchitis and hepatitis. Most of these effects are due to its anti-inflammatory and antioxidant activities, making it a good hepatoprotective plant. In addition, *C. lansium* stem bark with similar antidiabetic, anti-trichomonal and hepatoprotective activities as Curry Leaf would make a good substitute for it as a spicy vegetable. The reduction in these activities in *C. lansium* compared to that of Curry Leaf, may explain why *C. lansium* is commonly called Fool's or False Curry plant.

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