PHARMACOLOGICAL AND TOXICOLOGICAL ACTIVITIES OF THE METHANOLIC ROOT EXTRACT OF *CNESTIS FERRUGINEA* VAHL EX DE CANDOLLE (CONNARACEAE)

A THESIS SUBMITTED TO THE UNIVERSITY OF LAGOS, LAGOS, NIGERIA IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF DOCTOR OF PHILOSOPHY IN PHARMACOLOGY

By

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DECLARATION

This work titled ‘’Pharmacological and Toxicological activities of the methanolic root extract of *Cnestis ferruginea* Vahl ex De Candolle (Connaraceae)’’ submitted to the School of Postgraduate Studies, University of Lagos, Lagos, Nigeria for the award of Doctor of Philosophy in Pharmacology was original research carried out by Ishola, Ismaila Ogunbayode in the Department of Pharmacology, College of Medicine of the University of Lagos, under the supervision of Professor (Mrs.) O.O. Adeyemi, Dr. (Mrs) E.O. Agbaje and Dr. Rakesh Shukla.

The work has not been submitted previously, in whole or in part, to qualify for any other academic award.

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DEDICATION

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ABSTRACT

Cnestis ferruginea Vahl ex DC (Connaraceae) (CF) is a shrub widely used in traditional African medicine for the treatment of various painful inflammatory conditions and psychiatric disorders. The study was undertaken to investigate the toxicological profile and pharmacological effects with a view to isolate and characterize the active constituents of the methanolic root extract of CF responsible for these effects. Acute toxicity tests were carried out in mice and median lethal dose (LD_{50}) was estimated following oral and intraperitoneal administrations and as basis for dose selection in the pharmacological studies. Subchronic toxicity (90 days (plus 14 days reversibility)) studies were conducted in rats with oral daily doses of 100, 400 and 1000 mg/kg. Parameters observed for at the end of chronic tests include changes in body weight and vital organs weight, mortality, heamatological, biochemical, histological and oxidative stress parameters. Analgesic activity of the extract (100-400 mg/kg, *p.o.*) was evaluated using the acetic acid-induced writhing, formalin, tail clip and hot plate tests. The possible mechanism of its analgesic effect was investigated in mouse writhing and formalin tests using naloxone (μ- opioid antagonist), prazosin (α_1-adrenoceptor antagonist), ondansetron (5- HT_3 antagonist), haloperidol (D_2- receptor antagonist) and glibenclamide (ATP sensitive potassium channels). The anti-inflammatory effect was investigated using carrageenan-, egg albumin-, serotonin-, histamine-, and formaldehyde-induced rat paw oedema and xylene-induced ear oedema. Separation of phytochemical constituents and bioactivity guided assays were carried out with fractions on mouse writhing, and hot plate tests (analgesic effect) and carrageenan-induced rat paw oedema (anti-inflammatory effect). The maximal electroconvulsive (MES)-, strychnine-, picrotoxin-, bicuculline-, isoniazid-, and yohimbine-induced seizures were used to evaluate anticonvulsant effect in mice. The spectrum of activities of the extract on psychiatric disorders was studied using forced swimming and tail suspension tests to evaluate antidepressant effect while hole board, elevated plus maze (EPM), and light-dark tests were used to investigate anxiolytic effect. The involvement of 5-HT_2 receptor, α_1- and α_2-adrenoceptor, dopamine D_2 receptor, muscarinic cholinergic receptor, and nitric oxide pathway in the antidepressant effect of CF and CF-2 were investigated. Antidementic activity of the extract and its constituents were investigated using scopolamine-induced memory deficit in mice. Memory function was evaluated by passive avoidance and Morris water maze tests. Biochemical parameters of oxidative stress and cholinergic function were also estimated. The *in vitro* study was carried out to investigate the effect of CF-2 and CF-5 on

neuroinflammatory markers (oxidative stress, nitrative stress, and tumour necrotic factor-alpha (TNF-α)) in C6 and THP-1 cells respectively. Acute oral and intraperitoneal administrations of CF (>2 g/kg and >400 mg/kg respectively) produced behavioural signs of toxicity as well as mortalities within 24 h with estimated LD_{50} of 5.22 g/kg (*p.o.*) and 643.65 mg/kg (*i.p.*). In the subchronic test, CF at 100 mg/kg did not produce any significant irreversible deleterious effect on weight of animals and vital organs, *in vivo* antioxidants, heamatological, biochemical, sperm parameters and histological presentation. Platelet anomaly was elicited as delayed effect. The effects of the extract at 400 and 1000 mg/kg were similar but with delayed anaemia in females and weight reduction in males as side-effects. CF generally showed a potential to induce *in vivo* antioxidants enzymes. The methanolic root extract of *Cnestis ferruginea* (100, 200, and 400 mg/kg; *p.o.*) produced significant ($P < 0.05$) dose-dependent inhibition of pain response elicited by acetic acid and formalin while also increasing the nociceptive reaction latency in the tail clip and hot plate tests. The analgesic activity of the extract was significantly ($P<0.01$) reversed following naloxone, yohimbine, ondansetron, haloperidol, and glibenclamide pretreatment. In respect of anti-inflammatory activity, *Cnestis ferruginea* caused significant ($P < 0.05$) dose-dependent inhibition of oedema development in the carrageenan, egg albumin, serotonin, histamine, formaldehyde, and xylene-induced inflammation tests. The effects of the extract in the various models were generally comparable to those of the standard drugs used. Due to the promise shown by CF, an activity guided-isolation of active constituents was carried out which led to the isolation of amentoflavone (CF-2; a bioflavonoid) and an amino acid-like compound (CF-5) through column chromatography and spectroscopic methods (^{1}H NMR, ^{13}C NMR and HMBC). The methanolic root extract of *Cnestis ferruginea* (50-400 mg/kg, *p.o.*) produced significant ($P <0.05$) antagonism of MES-induced seizures and ameliorated the seizure induced by strychnine with peak effect observed at 50 mg/kg. CF produced significant ($P <0.05$) increase in onset of tonic convulsion which was comparable to the effect of clonazepam (0.5 mg/kg, *p.o.*). The extract produced 40, 20 and 20% protection respectively at 100, 200 and 400 mg/kg in picrotoxin-induced seizure. CF (100-400 mg/kg) completely antagonized bicuculline-induced seizure. Similarly, the extract produced dose-dependent increase in percentage protection in isoniazid and yohimbine-induced seizure in mice. Acute treatment with CF and its constituents significantly ($P < 0.001$) reduced the duration of immobility dose dependently in FST and TST. The pretreatment of mice with metergoline (4 mg/kg, i.p., a 5-HT_2 receptor antagonist) and reserpine (2 mg/kg, *i.p.*, a drug known to induce depletion of biogenic amines) 15 mins before the administration of CF (100 mg/kg; p.o.) significantly

prevented its antidepressant effect in the FST. However, pretreatment with prazosin (62.5 µg/kg, *i.p.*, an α_1-adrenoceptor antagonist), yohimbine (1 mg/kg, *i.p.*, an α_2-adrenoceptor antagonist), sulpiride (50 mg/kg, i.p., a dopamine D_2 receptor antagonist), atropine (1 mg/kg, *i.p.*, a muscarinic receptor antagonist) did not prevent this effect. The extract and its components produced significant (P <0.05) attenuation of anxiety shown by the increased number of head-dips in hole board, increased time spent in the open arms in EPM and increased exploration of light chamber. The anxiolytic effect of CF and CF-2 were reversed by flumazenil (3 mg/kg, *i.p.*) pretreatment. Scopolamine (3 mg/kg, *i.p.*) produced a decrease in transfer latency time (TLT) and an increase in escape latency time (ELT) in passive avoidance and Morris water maze tests respectively which are signs of memory deficits along with increased acetylcholinesterase (AChE) activity and oxidative stress in mice brain. Oral administration of CF, CF-2 and CF-5 significantly (P < 0.05) reversed scopolamine-induced memory impairments shown by the increased transfer latency time in passive avoidance test and decreased escape latency time in Morris water maze test. They also significantly (P <0.05) inhibited AChE and enhanced antioxidant enzyme activities in the brain following scopolamine injection as compared to vehicle administration in scopolamine (*i.p*)-treated mice which was comparable to effect of tacrine. Lipopolysaccharide (LPS) (10 µg/ml) stimulated C6 cells to release nitrite, reactive oxygen species (ROS), and malondialdehyde (MDA) while it down regulated glutathione (GSH) in C6 cells. Similarly, LPS up regulated the release of TNF-α in THP-1 cells. However, CF-2 and CF-5 significantly (P<0.001) attenuated nitrite release, ROS generation, MDA level and also up regulated the level of GSH. In addition, produced significant (P<0.05) attenuation of TNF- α level. CF-2 and CF-5, *per se* treatment did not have any significant effect on C6 and THP-1 cells. In conclusion, the methanolic root extract of *Cnestis ferruginea* given over an extended period is relatively safe and possesses significant analgesic, anti-inflammatory, anticonvulsant, antidepressant, anxiolytic, and antidementic effects, and these effects might have been produced by amentoflavone and/ amino acid like compound (CF-5) or combination of the phytoconstituents.