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Antidepressant and anxiolytic effects of amentoflavone isolated from *Cnestis ferruginea* in mice

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ABSTRACT

The root decoction of *Cnestis ferruginea* (CF) Vahl DC (Connaraceae) is used in traditional African medicine in the management of psychiatric disorders. This study presents the antidepressant and anxiolytic effects of amentoflavone (CF-2) isolated from the root extract of *C. ferruginea*.

The antidepressant effect was studied using the forced swimming (FST) and tail suspension tests (TST) while the hole-board, elevated plus maze (EPM) and light/dark tests were used to evaluate the anxiolytic effect. Acute treatment with CF extract and amentoflavone significantly ($p<0.001$) reduced the duration of immobility in FST and TST with peak effects observed at 100 and 50 mg/kg respectively in comparison to control treated. Antidepressant effects of CF and amentoflavone were significantly higher ($p<0.05$) when compared to imipramine in FST but comparable to the fluoxetine treated group in TST.

The pretreatment of mice with metergoline (4 mg/kg, i.p., a 5-HT2 receptor antagonist), prazosin (62.5 μg/kg, i.p., an α1-adrenoceptor antagonist), and yohimbine (1 mg/kg, i.p., an α2-adrenoceptor antagonist), but not sulpiride (50 mg/kg, i.p., a dopamine D2 receptor antagonist), cyproheptadine (3 mg/kg, i.p., a 5-HT2 receptor antagonist), atropine (1 mg/kg, i.p., a muscarinic receptor antagonist) 15 mins before the administration of amentoflavone (50 mg/kg; p.o.) significantly prevented its antiimmobility effect in the FST.

CF extract and CF-2 significantly ($p<0.05$) attenuated anxiety by increasing the number of head-dips in the hole-board test, the time spent on the open arms in the EPM, and the exploration of the light chamber in the light/dark test. Pretreatment with flumazenil (3 mg/kg, i.p., ionotropic GABA receptor antagonist) 15 min before oral administration of amentoflavone (25 mg/kg) significantly reduced the time spent in the open arms in EPM. It is concluded from the results obtained that amentoflavone produces its antidepressant effect through interaction with 5-HT2 receptor and α1-, and α2-adrenoceptors while the anxiolytic effect involved the ionotropic GABA receptor.

1. Introduction

Cnestis ferruginea Vahl ex DC (Connaraceae) is a short ornamental shrub, sometimes a climber, which is about 2.5 meters high and is usually covered by dense, brown velvety hairs (Hutchinson and Dalziel, 1958). The plant is widely employed in the treatment of various ailments in traditional medicine throughout West Africa. The root is used in south-western Nigeria for all manners of pains, mange, asthenia, as purgative and as a sedative in insanity (Burkhill, 1985). The root decoction is taken by draught in Ivory Coast and Upper Volta (now Burkina Faso) as an aphrodisiac, and by enema for gynaecological troubles, dysentery and urethral discharge (Burkhill, 1985). The antistress, antinociceptive and anti-inflammatory properties of the root extract of *C. ferruginea* have previously been reported (Ishola and Ashorobi, 2007; Ishola et al., 2011).

The findings in these previous studies justify the need to isolate the active principle(s) responsible for anti-inflammatory and central nervous system effects of the extract. Through an activity guided fractionation assay, amentoflavone (a bioflavonoid) was isolated (Ishola et al., 2012). Identification and structural elucidation was carried out through series of spectroscopic techniques (^{1}H, ^{13}C NMR, HMBC, HSQC and mass spectra (*m/z*)). The spectral data were compared to the published data of amentoflavone (Ishola et al., 2012; Markham et al., 1987).

Flavonoids are naturally occurring polyphenolic compounds that have many biological properties, including antioxidative, anti-inflammatory and neuroprotective effects. Amentoflavone is a unique biflavonoid consisting of an apigenin dimer. The neuroprotective

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effect of amentoflavone is evident in its ability to reduce cell death induced by staurosporine, etoposide and sodium nitroprusside in neuroblastoma SH-SY5Y cells (Shin et al., 2006). In addition, it markedly reduced the hypoxic-ischeamic induced brain tissue loss with a wide therapeutic time window, up to 6 h after the onset of hypoxia. Amentoflavone blocked the activation of caspase 3, characteristic of apoptosis, and the proteolytic cleavage of its substrates following hypoxic-ischemic injury (Back et al., 2002). Treatment of mouse microglial cells with amentoflavone resulted in a significant decrease in the lipopolysaccharide-induced production of nitric oxide and induction of inducible nitric oxide synthase and cyclo-oxygenase-2. Furthermore, amentoflavone decreased the inflammatory activation of microglia after hypoxic-ischeamic injury when assessed by the microglia-specific marker (Shin et al., 2006).

Previous studies have shown that amentoflavone bind to brain benzodiazepine receptors with an affinity comparable to diazepam (Nielsen et al., 1988; Baureithel, et al., 1997). Amentoflavone has been found to be taken up into porcine brain endothelial cells predominantly by passive diffusion and is transported across porcine brain capillary endothelial cells (BCEC) monolayer, suggesting that amentoflavone would be able to penetrate the brain in vivo (Gutmann et al., 2002). A comprehensive battery of in vitro radio-ligand binding assays has also shown that amentoflavone significantly inhibits binding at serotonin (5-$HT_{1D\alpha}$ $K_i = 4094$ nM, 5-HT_{2C} $K_i = 2555$ nM), D_3-dopamine ($K_i =$ 1241 nM), and δ-opioid receptor subtype ($K_i = 36.5$ nM) (Hanrahan et al., 2003).

On the basis of these considerations, this study was carried out to evaluate the effect of amentoflavone and *C. ferruginea* against anxiety and depression in mice. We also investigated the involvement of monoaminergic and cholinergic systems in the antidepressant-like effect of amentoflavone so also the involvement GABAergic system in anxiolytic effect of amentoflavone.

2. Materials and methods

2.1. Plants material

The dried roots of *C. ferruginea* were purchased from a traditional herbal practitioner in Mushin, Lagos State, Nigeria. The botanical identification and authentication of the plant were done by Mr. Joseph Ariwaodo of the Forestry Research Institute of Nigeria (FRIN), Ibadan, Nigeria and Prof. J.D. Olowokudejo of the Department of Botany and Microbiology, Faculty of Science, University of Lagos, Lagos, Nigeria respectively. A voucher specimen of the plant was deposited at the herbarium of the Institute (FHI 108219).

2.1.1. Preparation of extract

Powdered root of *C. ferruginea* (5.2 kg) was loaded into a glass percolator containing methanol (20 L). It was allowed to stand at room temperature (28 °C) for about 16 h (overnight). The percolate was collected and the process of extraction was repeated five times. The combined extract was filtered and concentrated on Buchi Rotavapor at 40 °C. It was further dried under vacuum pump. The weight of the brownish extract obtained was 560 g (10.77% yield).

2.1.2. Isolation and identification of active compounds

The dried extract was suspended in water followed by successive partitioning with $CHCl_3$, EtOAc, n-butanol respectively. The aqueous/n-butanol fraction (160 g) was chromatographed on silica gel column, eluted with a gradient of $CHCl_3$: MeOH (100:00–00: 100). Fractions with similar R_f were pooled together affording seven subfractions. Fr.4, being the most active, was rechromatographed as described above with addition of 5% H_2O, given amentoflavone (CF-2) (350 mg) and an amino acid (2.2 g) whose structure is still under investigation. CF-2 structure was identified by spectral data (IR, ^1H NMR and ^{13}C NMR, HMBC, HSQC), which was in accordance with those previously described (Ishola et al., 2012; Markham et al., 1987).

2.2. Laboratory animal

Naive 8 weeks old male Swiss albino mice (20–30 g) were obtained from the Laboratory Animal Services Division of Central Drug Research Institute, Lucknow. The animals were housed in groups of six mice per cage in a controlled environment (room temperature 24–27 °C and humidity 60–65%) with a 12 h light and dark cycle (lights on 0700 hours) in a pathogen-free colony. Food, in the form of dry pellets, and water were freely given ad libitum. The care and use of animals were conducted in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals (2011). This study was approved by the research ethics committee of Central Drug Research Institute and CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals). Mice were kept in the colony 1–2 weeks before the experimental procedures. Behavioural sessions were conducted between 0900 hours and 1400 hours.

Healthy mice were screened on the basis of the swimming ability and normal behaviour. Rotarod test in mice (before and after administration of drug) was used to validate muscle co-ordination activity of mice. Mice, showing the normal fall time of 2 min from rotating rod at 20 rpm speed, were selected for forced swimming test. Animal showing abnormal muscle coordination before or after drug administration was excluded from the study.

2.3. Drugs and treatment regimens

All drugs were freshly prepared before use and administered in a volume of 10 ml/kg. Drug doses were calculated as the base weight and expressed as milligram per kilogram. The following drugs were used: Sulpiride (50 mg/kg, i.p.), flumazenil (3 mg/kg, i.p.), prazosin (62.5 μg/kg, i.p.), atropine (1 mg/kg, i.p.), cyproheptadine (3 mg/kg, i.p.), yohimbine (1 mg/kg, i.p.), and metergoline (4 mg/kg, i.p.) (Sigma Aldrich, St. Louis, MO, USA). The drugs were administered to mice 24 h after the pretest and 15 min before the test and reference drugs; fluoxetine (20 mg/kg, i.p.), diazepam (1.5 mg/kg. p.o.) (Sigma Aldrich, St. Louis, MO, USA), and imipramine hydrochloride (20 mg/kg; p.o.; Dalkeith Laboratories Limited, Woburn, MK17, UK). All drugs/extract were dissolved in deionised water suspended in gum acacia (0.5%). Graded doses of *C. ferruginea* (25–200 mg/kg, p.o.), amentoflavone (6.25–50 mg/kg, p.o.), and imipramine (20 mg/kg, p.o.) were administered to mice 1 h prior to the forced swimming test (FST). The most effective dose was used to evaluate the mechanism(s) of action(s). The effective dose identified in FST was utilised in tail suspension test model to confirm their antidepressant activity using fluoxetine (20 mg/kg, p.o.) as standard antidepressant drug.

2.4. Behavioural observations

Mice were randomly divided into four groups of 8 mice each: Group I – vehicle (10 ml/kg), and Groups II–IV – *C. ferruginea* (100, 200 and 400 mg/kg). The mice were subjected to spontaneous motor activity and neuromuscular coordination tests.

2.4.1. Spontaneous motor activity in mice

Gross open field activity was studied using digiscan infrared photocell system (test box model: RXYZCM (16 TAO); Omnitech Electronics, Columbus, Ohio) in 42×42×30 cm Plexiglass arenas, fitted into infrared beam containing metallic grid. Activity of animals was observed by the interruptions of infrared beams.

Horizontal activity—the total number of beam interruptions that occurred in the horizontal sensor within 2 min.