Anti-nociceptive and anti-inflammatory effects of the hydroethanolic extract of a polyherbal preparation (Cov-Pla 2) in laboratory animals

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Abstract
Symptoms of Coronavirus Disease-2019 include pulmonary hyper-inflammation; managing local and systemic inflammatory responses may be key in treatment. This study evaluated the anti-inflammatory, analgesic and antipyretic effects of a mixture (Cov-Pla 2) containing five medicinal plants in laboratory animals. The anti-nociceptive activity of Cov-Pla 2 was evaluated using acetic acid-induced writhing, Brewer’s Yeast Pyrexia test in rats and hot plate tests in mice. The egg albumin-induced rat paw oedema test was employed to evaluate the extract’s anti-inflammatory activity. The extract produced a dose-dependent (125-500 mg/kg, p.o.) inhibition of pain response elicited by acetic acid, compared to normal saline and increased reaction latency in the hot plate test. The anti-inflammatory test showed a significant (P<0.05) reduction in paw size diameter at 125 mg/kg from 2 hours, compared to control. In the antipyretic test, the extract produced a significant (P<0.05) time-dependent decrease in rectal temperature at 125 mg/kg after 2 and 3 hours and at 250 and 500 mg/kg after 3 hours, compared to normal saline. The extract did not produce mortality up to 5000 mg/kg per oral. The results indicated that the extract possesses anti-nociceptive and anti-inflammatory properties, which support its recommendation for trials in the treatment of symptoms associated with COVID-19.

Keywords: COVID-19; Inflammation; Nociception; Polyherbal; SARS-CoV-2
INTRODUCTION

Coronavirus disease-19 (COVID-19) caused by Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2) has caused a pandemic that has overwhelmed health care systems globally [1,2]. Currently, no specific effective treatment for COVID-19 exists. COVID-19 is characterized by a wide spectrum of symptoms including abdominal pain, fever, cough, headache, diarrhea, tiredness, runny nose, sore throat and vomiting, while loss of smell or taste is experienced by some people [3, 4]. Respiratory problems such as shortness of breath or difficult breathing, viral pneumonia and respiratory failure have also been reported. Recently, an inflammatory syndrome has been reported in some patients [5]. SARS-CoV-2 is reported to trigger a cell death-induced “eicosanoid storm”, that involves prostaglandins and leukotrienes, which in turn initiates a robust inflammatory response [6].

The global public health threat caused by the coronavirus disease ignited a lot of scientific search for a cure. This resulted in several formulations, one of which is Cov-Pla 2. This formulation contains parts of 5 plants African ethnopharmacological relevance with bioactivity against microbes, including viruses [7]. Polyherbal preparations offer disease therapy a near holistic approach [8]. This crucial traditional therapeutic herbal approach involves combining several medicinal herbs to accomplish desired therapeutic efficacy [9]. The concept of Ayurvedism that wellness depends on a delicate balance of the mind, body and spirit provides that in order to obtain the desired therapeutic effect, different plant phytochemicals need to be combined, as the active phytochemical constituents of individual plants may be insufficient [9].

Plants, containing medicinal compounds or phytochemicals, have been used for different purposes over time, including the relief of pain and inflammation [10, 11]. Considering the fact that analgesic drugs like salicylic acid and morphine were originally derived from plants, the utilization of plants with anti-nociceptive and anti-inflammatory properties for treating diseases with symptoms of pain and inflammation would be considered a productive strategy.

The aim of this study was to evaluate the anti-nociceptive and anti-inflammatory activities of the hydroethanolic extract of Cov-Pla 2 polyherbal preparation in rodents.

EXPERIMENTAL

Test animals. Three-week old albino rats (140-200 g) and mice (20-30 g) of both sexes were purchased from the Animal Experimental Unit, Department of Pharmacology and Toxicology, University of Jos, Nigeria. The animals were kept at room temperature (27 ± 2°C; 70-80 % humidity; 12 h light/dark cycle) in the Animal Experimental Unit, Department of Pharmacology and Toxicology, University of Jos, Nigeria for at least 48 h prior to the procedure. Commercial food pellets (Livestock Feeds, Nigeria Ltd) and water were supplied ad libitum. Animal experimental protocols were in accordance with the current guidelines for the care and use of laboratory animals [12]. Approval to conduct the study was given by the Animal Ethics Committee of the Department of Pharmacology and Toxicology, University of Jos, Nigeria under the ethical certificate number F17.00379 dated 5th June, 2020.

Drugs and Chemicals. Distilled water, Normal saline 0.9 % w/v, Diclofenac Sodium (Novartis, 20 mg/kg), Morphine (Sterop Pharma, Belgium), Acetic acid 0.6 % (M&B), Formalin 1 % (B&B), Brewer’s Yeast (ICH), Acetylsalicylic acid 300 mg (Sigma) and Egg white from eggs purchased at a local poultry.

Collection and preparation of plant materials. The plants were identified by Mr. Joseph Ajila, a taxonomist at the Federal College of Forestry, Jos, Nigeria. Voucher
specimens were deposited at the herbarium of the institute. One thousand grams (1000 g of the dried parts of the different plants were then pulverized, mixed together and macerated in 10 L of hydroethanol (3 liters water to 7 liters 70 % ethanol) for 24 h. The mixture was thereafter decanted, filtered and the residue was re-macerated in 1000 mL hydroethanol for 24 h. This process was repeated two more times to ensure exhaustive extraction. At the end of the extraction process, the combined filtrate was evaporated to dryness under reduced pressure at 40°C. A dark brownish solid extract with a yield of 15.4 % was obtained. The solid extract was reconstituted in distilled water to give appropriate concentrations before administration to experimental animals.

**Acute toxicity test (LD$_{50}$ Determination).** The method of Lorke was used to estimate acute toxicity [13]. Briefly, three groups containing three mice each were fasted for 12 h before the test and administered the extract orally at the dose of 10 mg/kg, 100 mg/kg and 1000 mg/kg respectively. The mice were observed over a period of 24 h for morbid signs and mortality. In the second phase, another three animals were divided into three groups with one animal each and administered 1600 mg/kg, 2900 mg/kg and 5000 mg/kg of the extract respectively and then observed for 24 h for behavior as well as mortality.

**Screening for anti-inflammatory activity.**

(a) **Egg albumin-induced rat paw oedema test.** Treatment with normal saline (10 mL/kg, p.o.), standard drug, diclofenac (20 mg/kg) and extract (125, 250, 500 mg/kg, p.o.), were administered to five groups of five rats each. One hour post-treatment, oedema was induced by injection of egg albumin (from fresh hen's egg) (0.1 mL, 0.01 g/mL saline) into the sub-plantar tissue of the right hind paw. The linear paw circumference was then measured using a digital vernier caliper. Linear paw circumferences of the rats were determined just before injection of the phlogistic agent and at 30-min intervals for 3 h [14]. The percent inhibition of inflammation was calculated using the formula [15]:

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\text{% inhibition} = 100\left(1 - \frac{\text{Vt}}{\text{Vc}}\right),
\]

where \(\text{Vc}\) represents oedema volume in control and \(\text{Vt}\) the oedema volume in the test groups.

**Screening for antinociceptive activity**

(a) **Acetic acid induced writhing in mice.** Mice (20-30 g) fasted overnight were divided into five groups of five animals each. The animals were then treated with normal saline 0.9 % w/v (10 mL/kg, p.o.) (control group), extract (125, 250, 500 mg/kg, p.o.) and diclofenac (20 mg/kg, s.c.). Thirty minutes post oral and subcutaneous administration; test animals were administered acetic acid (0.6 % v/v, 0.1 mL/kg, i.p.). The number of writhes (characterized by contraction of the abdominal musculature and extension of the limbs) was then counted for 20 minutes at 5-minute intervals [16].

(b) **Hot plate test in mice.** Albino mice used in this experiment were initially screened by placing the animals in turn on a hot plate (Stuart Model US150) set at 50 ± 0.5°C [17], and animals which failed to lick the hind paw or jump (nociceptive responses) within 15 s were not used for this study. Eligible animals (20-30 g) were divided into five groups of five mice each, and the pretreatment reaction time for each mouse was determined. Mice in different groups were then treated with normal saline 0.9 % w/v (10 mL/kg, p.o.) (control group), extract (125, 250, 500 mg/kg, p.o.), and morphine (1 mg/kg, s.c.) (standard drug). The reaction time of each mouse was then determined at 5 minutes before administration of test agents and 30, 60, and 90 minutes after administration. A cut-off time of 30 s was used [18].

(c) **Antipyretic test in rats (Brewer's yeast pyrexia model).** Antipyretic activity in albino rats was evaluated with induction of fever by 20 % Brewer yeast (1 mg/kg). Albino rats (140-200 g) appropriately restrained had their rectal temperature measured by inserting a digital thermometer into the rectum. Pyrexia
was induced by subcutaneously injecting 1 mg/kg of a 20% suspension of Brewer yeast. After 18 h of yeast injection, the rats which showed a rise in temperature of at least 1°F (0.6°C) were taken for the study. Twenty-five of the rats that showed this temperature increase were then divided into five groups (n = 5). Animals in group 1 received normal saline 0.9% w/v (10 mL/kg, p.o.), group 2 had the standard drug, aspirin (300 mg/kg p.o.), and groups 3, 4 and 5 received the extract (125, 250, 500 mg/kg, p.o.). Temperatures were measured and recorded 5 minutes before and 1 h, 2 h, 3 h and 4 h post-extract, aspirin and normal saline administrations [19].

**Statistical analysis.** Data are presented as mean ± Standard Error of the Mean (SEM). Statistical analysis was done using Student's t-test and two-way ANOVA, using GraphPad Prism 5 software (GraphPad Software, Inc., La Jolla, CA, USA). Results were considered significant between test groups and control at \( P<0.05 \).

**RESULTS**

**Acute toxicity testing (LD\textsubscript{50} determination).** Acute toxicity testing showed that the extract did not cause any mortality up to 5000 mg/kg (Table 1).

**Anti-inflammatory effect of hydroethanolic extract of Cov Pla-2 on egg albumin-induced inflammation in rats.** Results in table 4 below show a reduction in paw circumference (\( P>0.05 \)) from 1.5 h up to 3 h post-administration at 125-500 mg/kg, compared to animals administered normal saline.

**Effects of the hydroethanolic extract of Cov-Pla 2 on acetic acid-induced abdominal writhing in mice.** Results showed that the extract at all doses (125-500 mg/kg) caused a decrease in number of writhing, compared to animals administered normal saline. Diclofenac (a standard drug) produced a significant decrease (\( P<0.05 \)) compared to control (Table 2).

**Effects of hydroethanolic extract of Cov-Pla 2 on reaction time in the hot plate test in mice.** The result in table 3 shows a dose-dependent increase (250-500 mg/kg) in reaction time after 60 and 90 minutes post-administration, compared to the animals administered normal saline.

**DISCUSSION**

The safety evaluation of medicinal plants in laboratory animals is a useful tool in predicting their toxicity in humans. In this present study, no mortality was observed up to 5000 mg/kg body weight in test animals (Table 1), which is an indication of its relative safety at the doses used.

The writhing test is simple, reliable and affords rapid evaluation of antinociceptive activity [20]. The dose-dependent (125-500 mg/kg) inhibition of the acetic acid induced writhing by the extract (Cov Pla-2) is suggestive of its antinociceptive activity, which might be peripherally mediated. This is based on the association of the model with stimulation of peripheral receptors, especially the local peritoneal receptors at the surface of cells lining the peritoneal cavity [21]. Result of the writhing test from this present study corresponds to the report of a previous study [22]. The abdominal constrictions response induced by acetic acid is a sensitive procedure to evaluate peripherally acting analgesics. The acetic acid induced writhing test is useful for the evaluation of mild, anti-nociceptive, non-steroidal anti-inflammatory drugs [23]. Acetic acid causes pain generally by releasing endogenous substances such as serotonin, histamine, prostaglandins, bradykinins and substances, which stimulate nerve endings.
Therefore, the analgesic activity of the extract may be peripherally mediated via the inhibition of synthesis and releases of prostaglandins. Pain induced by thermal stimulus of the hot plate is specific for centrally mediated nociception [24]. The authors from that study found that the ability of an extract to increase the reaction latency to thermally induced pain in mice by the hot plate is an indication of its central analgesic activity.
Findings from this present study correlated with the findings of [23], where reaction time was seen to increase in a dose dependent (125-500 mg/kg) manner over time.

The anti-inflammatory activity of the polyherbal extract (Cov Pla-2) was evaluated in this study using the egg albumin rat paw oedema test. The paw size of all the treated groups in this test decreased from the first hour post-oedema induction in a dose-dependent manner (125-500 mg/kg) as compared to the control group (Table 4). Oedema reduction in the treated groups increased with time, a finding that is supported by a previous study involving inhibition of egg albumin-induced oedema by an extract [25]. Our extract may have elicited its anti-inflammatory effect by inhibiting the release of histamine and 5-HT, two mediators that are released by egg albumin [26].

The rats treated with the extract showed a reduction in rectal temperature in a dose-dependent (125-500 mg/kg) manner as early as 2 h post hyperthermia-induction (Table 5), when compared with control animals. The ability of an extract with antipyretic activity to also reduce rectal temperature, as also shown in this present study, was similarly shown in an earlier study [27]. These results support the use of the extract as an antipyretic for the treatment of fever.

Conclusion. This study demonstrated the antinociceptive, anti-inflammatory and antipyretic properties of the hydroethanolic polyherbal extract using several models (chemical and thermal) of nociception in mice and rats. The findings suggest their possible use to treat inflammation and pain associated with Covid-19 disease.

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