JOPAT Vol 20(2) 624– 639, July – Dec. 2021 Edition.

ISSN2636 – 5448 https://dx.doi.org/10.4314/jopat.v20i2.1

COVID ORGANICS as herbal remedy for treatment of COVID-19: Evaluation of oral safety, antitussive, expectorant and antipyretic activities

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Abstract

COVID ORGANICS (CVO) is a herbal remedy developed for treatment of COVID-19. This study was aimed to verify oral safety and efficacy the product against symptomatic presentation of COVID-19 infection. Its acute oral safety was investigated using the limit test (2000 mg/kg, p.o.) in mice, while the sub-chronic toxicity was investigated by 28 days daily administration of CVO (1000 mg/kg) in rats. The antitussive, mucosecretolytic and antipyretic activities of three dose levels of the product (50, 200 and 800 mg/kg, p.o.) were evaluated using standard models in rats and mice. Results showed that the product was safe, exhibited suboptimal antitussive, expectorant activities and reduced pyrexia at higher doses (200 and 800 mg/kg, p.o.).

Key words: COVID ORGANICS; oral safety; antitussive; expectorant; antipyretic

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Introduction

COVID-19 is a pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). The virus is spread primarily via nose and mouth secretions including small droplets produced by coughing, sneezing, and talking.

Most common symptoms are fever, dry cough and tiredness, while the less common symptoms include aches and pains, sore throat, diarrhea, conjunctivitis, headache, loss of taste or smell, a rash on skin, and discolouration of fingers or toes. Complications arising from being infected with the disease include pneumonia and acute respiratory distress syndrome. The time from exposure to onset of symptoms may range from two to fourteen days. Despite some success achieved through the use of vaccines, the disease still ravages different countries with fatal outcomes in some cases. In an event of infection, current approach for medical management is largely supportive since no targeted therapy is available yet. Thus, any agent that can effectively reduce the symptoms associated with infection would be ideal for evaluation.

COVID ORGANICS (CVO) is a herbal remedy for treatment of COVID-19 donated by the Republic of Madagascar to the Federal Republic of Nigeria. The product is claimed to strengthen the immune system, treat viral infections, reduce fever and cure breathing difficulties. The producers of CVO however make no anti COVID-19 claim. It has been recognized that cough is refractory to specific, single therapy in a significant number of patients [1], even when an underlying cause has been identified [2]. This study is primarily aimed at evaluating oral acute and sub-chronic toxicities and insight into its pharmacological effects in symptomatic relief of COVID-19 infection by investigating its antitussive, respiratory tract mucus expelling capability and antipyretic, using standard models in laboratory animals.

Materials and Method

Test material

COVIDORGANICS (CVO) Tambavy Tisane Herbal Tea (sample no: 01/03/05062020/ 556/0180 and 01/03/05062020/556/0181) is a sachet containing mixture of 62% *Artemesia annua* and 38% other Malagasy herbs weighing ~ 16.5 g each. It was manufactured on 28th April 2020 with a one year expiring period from the manufacturing date. The product is indicated to strengthen the immune system, treat viral infections, reduce fever and cure breathing difficulties. It is prepared by infusing 1 sachet of the product in 1 L of hot water for 15 min. It is then filtered and used within 24 h. Adults receive 33 cl twice daily; 11 – 15 years, 33 cl daily; 6 – 10 years, 33 cl every two days; while 2 – 5 year's receive 15 cl every two days. The product is not recommended for children under 2 years old, pregnant and lactating women and those with impaired kidney and hepatic function.

Preparation of test agent

The test agent was prepared by infusion to mimic CVO's ethnopharmacy. Three (3) sachets of the material were each soaked in 1 L of boiled distilled water for 15 min; in multiples. The extracts were be pooled together, filtered, and the filtrate dried in an oven to obtain infusion extract of CVO. The extract was

stored in an amber glass container and kept in a refrigerator until use.

Animals

Wistar rats and Swiss albino mice of both sexes for the study were bred at the Animal Facility Centre (AFC) of National Institute for Pharmaceutical Research and Development (NIPRD), Abuja. They were used following the International Guiding Principles for Biomedical Research Involving Animals [3]; in line with NIPRD's standard procedures on laboratory animal usage (NIPRD/05:3:06 – 07).

Oral acute toxicity test

The oral acute safety of CVO was evaluated by Limit Test using the modified Acute Oral Toxicity–Up-and-Down-Procedure (UDP) of Organization for Economic Cooperation and Development (OECD) Test No. 425 [4], in rats. Rats were acclimatized individually in metabolic cages (Techniplast, Buguggiate, Italy) used for the study for 3 days. Prior to the test day, the rats were subjected to an overnight fast, with free access to drinking water. On the test day, the rats were randomized by weight keeping them mean weight as near as possible into 4 groups of 3 rats each. In Group 1, 3 male rats were given CVO, corresponding to 2000 mg/kg doses, while in Group 2, 3 male rats received the vehicle (distilled water, 10 mL/kg) to serve as the male control group. Group 3, 3 female rats were given CVO, corresponding to 2000 mg/kg doses, while in Group 4, 3 female rats received the vehicle (distilled water, 10 mL/kg) to serve as the female control group. All the rats were systematically observed for signs and symptoms of toxicity using established criteria adopted from Malone and Robichaud [5] Hippocratic screening table.

The criteria used were: central nervous system (CNS) stimulating (> motility, > respiration, tail erection, > stereotype, paw licking, mouth scratching, convulsion, tremors); CNS depressing (< motility, < respiration, analgesia, anaesthesia, corneal reflex, paralysis, sedation), and others parameters that included; excess urine, ear pallor, hair erection, diarrhoea, writhing, passivity, jumping, retching, rearing and mortality. Thereafter, the animals' weight changes, water intake, food consumption, excretion of faeces and urine were measured every 2 days for 14 days.

Sub-chronic toxicity evaluation

The sub-chronic toxicity was evaluated in rats using the Repeated Dose 28-Day Oral Toxicity Study in Rodents, (OECD) Test No. 407 [6]. The rats were randomized by weight keeping the mean weight as near as possible, and then grouped into four groups of 5 rats in the following manner: Group 1, male (treatment); Group 2, male (control); Group 3, female (treatment); and Group 4, female (control) rats. The rats were first acclimatized individually in metabolic cages (Techniplast, Buguggiate, Italy) used for the study for five days. The treatment groups received 1000 mg/kg, p.o. daily for 28 days, while the control received the vehicle (distilled water, 10 mL/kg) also daily for 28 days. The animals' body weights, water intake, food consumption, feces,

and urine output were measured occasionally (D_3, D_7, D_{14}, D_{21}, and D_{28}) throughout the observation period. On day 29, blood was collected for hematological and biochemical analysis. The hematological parameters were evaluated using an automated haematology analyser YNH7021 (Wincom Company Ltd. Hunan, China); and the parameters evaluated were red blood cells, haemoglobin, haematocrit, mean corpuscular volume (MCV); mean corpuscular haemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets, total leukocytes, neutrophils, lymphocytes and monocytes–basophils–eosinophils mixed (MXD).

The biochemical analysis were performed using appropriate assay kits from: Randox Laboratories Ltd, Crumlin, United Kingdom; Agape Diagnostics, Cham, Switzerland; Spectrum Diagnostics, Hannover, Germany; Teco Diagnistics, Anaheim, USA: and Fortress Diagnostics Ltd, Antrim, United Kingdom. The parameters tested were glucose, urea, creatinine, uric acid, alanine amino transferase, aspartate amino transferase, alkaline phosphatase, total cholesterol, triglycerides, high-density lipoproteins and total proteins, gamma-glutamyl transferase, albumin, direct bilirubin, total bilirubin chloride and potassium ions.

Thereafter the animals were humanly euthanized and the internal organs such as brain, liver, spleen, left kidney, right kidney, heart, stomach and testes (male) or uterus (female) were removed and their relative organ weight analysed - by dividing each animal's organ weight by their body weight.

Antitussive activity

The ability of CVO to suppress cough was evaluated in mice using the ammonia vapor-induced model described by Wang *et al*. [7]. Mice were allotted into five groups (n = 5) keeping their mean weight as near as possible. Groups 1 was treated orally (p.o.) with the vehicle (10 mL/kg) to serve as the negative control; Groups 2 – 4 were treated orally with CVO (50, 200 and 800 mg/kg, p.o.); while Group 5 received dihydrocodeine (30 mg/kg) to serve as the standard control. An hour after the treatment, each mouse was placed in a 10×10×10 cm glass chamber and exposed to 0.3 ml of 25% ammonia vapor for 1 min. The mouse was removed from the chamber thereafter and placed in a 1000 mL beaker. The number of cough bouts exhibited by each mouse within 5 min was counted. The criteria used to define cough in mice were: opening of the mouth with contraction of thoracic and abdomen muscles and jerking of the front body. Antitussive activity was assessed as a percentage of inhibition of the number of cough bouts relative to the control group as follows:

Inhibition (%) = [(Co−Ct)/Co×100%]; Where Co: the number of cough bouts of control, Ct: the number of cough bouts of the treatment group.

Expectorant activity

This was determined using an optimized model [9]. Fifty mice were randomized based on body weight into five groups, I–V, (n = 10). The mice were pre-treated daily for 5 days as follows: Group I, 10 mL/kg vehicle; Group II-IV, CVO 50, 200 and 800 mg/kg; while group 5 received ammonium chloride (500 mg/kg) as the positive control. Thirty minutes after treatment on the 5th day, mice were intraperitoneally injected with 500 mg/kg phenol red from a 1.25%w/v solution prepared in normal saline. After 30 min, the mice were euthanized by chloroform inhalation, The tracheal portion extending from the thyroid cartilage to the main stem bronchi was excised from each mouse and cleared of adhering tissue, taking care to minimize contamination with blood. Each excised tracheal tissue was washed in 2 ml normal saline by gentle vortex. Sodium hydroxide (160 µL, 0.1 M) was added to 1.6 mL of each washing to stabilize pH. The absorbance of the washings was taken at a wavelength of 565 nm using a spectrophotometer (Cary 60, Agilent technologies). Concentrations of phenol red in lavage fluid were determined from a phenol red calibration plot. The calibration plot was prepared using 1 mg/mL stock solution of phenol red prepared in normal saline. A 200 µL volume of this stock was diluted with to 9.8 mL normal saline to give 10 mL of 20 µg/mL solution. This solution was serially diluted to give 10, 5, 2.5, 1.25 and 0.625 µg/mL serial dilutions. A volume of 1.6 mL of each dilution was stabilized with 160 µL of 1 M sodium hydroxide solution, and the absorbance read at 565 nm.

Antipyretic activity

The lipopolysaccharide-induced (LPS)-induced fever method described by Santos and Rao. [8] was used for the assay. This experiment was performed during the light phase of the circadian cycle, between 9 am and 5 pm. Forty-eight rats were randomly divided into six groups (n = 8) and fasted overnight for 18 h, but allowed free access to drinking water. After an 18 h fast, rats in Groups 1, 2, 3 received 50, 200 and 800 mg/kg body weight of CVO by gavage. In Group 4, each animal received 10 mg/kg body weight of indomethacin orally. Animals in Group 5 and 6 served as hyperthermic and normothermic controls respectively and received the aqueous vehicle (pyrogen-free water) orally. After 1 h the animals in Group 6 received pyrogen-free sterile water for injection (Juhel Nigeria Ltd.) via intraperitoneal injection (10 mL/kg), while the other groups (1 -5) were injected intraperitoneally with LPS (100 µg/kg) dissolved in pyrogen-free sterile water for injection. Body temperature (Tb) was taken every 30 min for 3 h after LPS administration. Body temperature was measured by gently inserting a small digital thermometer about 3 cm into the rectum until a beep sound heard. During the temperature measurements, each animal was held gently during the experiment to avoid changes in rectal temperature secondary to handling.

Data Analysis

Results were expressed as mean ± SEM. Data were analyzed by one-way analysis of variance (ANOVA) followed by Student-

Newman-Keuls test using Graph-Pad Prism for Windows Version 6.0 (Graph-Pad Software, San Diego, CA, USA). Differences between means of treated groups and the control were regarded as statistically significant at $p \leq 0.05$. For antipyretic assay, temperature difference for each rat was obtained by subtracting the respective basal levels from each subsequent value at 0.5, 1, 1.5, 2, and 3 h. The area under the temperature difference-time response curves (AUC) for each rat was also determined by trapezoidal integration using MS Excel.

Result

CVO extract physiochemical parameters

The resultant infusion extract of CVO gave the following physicochemical parameters: yield = 25.28% w/w; colour = dark brown; and solubility >500 mg/mL in distilled water.

Acute toxicity test

No death was recorded in CVO treated rats (2000 mg/kg, p.o.) throughout the 14 days observation. The agent initially produced mild CNS stimulatory activity characterized by paw licking and mouth scratching within 30 min in both the male and female groups, which reversed after 1 h in relation to control. None of the animals presented behavioural changes or clinical signs and symptoms of toxicity.

Determination of biophysical parameters (body weight, water intake and food consumption and excretions of faeces and urine)

The daily dosing of CVO (1000 mg/kg) did not cause significant changes in body weight, water intake, food consumption and excretion of faeces when compared to the vehicle group (Table 1). There was an increase excretion of urine in male group by the 3rd day (D_3) ($p < 0.05$) and 14th Day (D_{14}) which normalised on D_7 and D_{21} respectively, when compared to the vehicle group. There was also a significant water intake in female group by D_3 that normalised on D_7.

Haematological parameters

Treatment with CVO (1000 mg/kg, p.o.) for 28 days did not significantly alter most of the parameters investigated. It however presented a decrease ($p < 0.05$) in the absolute value of platelets counts in the male group when compared to the vehicle group. All the other parameters were within normal range in relation to the vehicle (Table 2).

Biochemical parameters

Daily treatment with CVO (1000 mg/kg, p.o.) for 28 days did not significantly alter all the parameters investigated in the male group. However, significant increase in alkaline phosphatase was observed in the female group in relation to the vehicle (Table 3).

Gross and relative organ weights

The treatment of rats with CVO (1000 mg/kg, p.o.) for 28 days did not cause macroscopic changes in the organs analysed. Also, no significant changes were observed in

the relative organ weight indices in all the tested animals in relation to the vehicle group (Table 4).

Table 1: Effect of 28 days oral administration of CVO (1000 mg/kg, p.o.) on body weight, water intake and food consumption, and excretion of faeces and urine in male and female rats.

Parameter	Period of treatment (Days)					
	D_0	D_3	D_7	D_{14}	D_{21}	D_{28}
Male (CVO 1000 mg/kg)						
Body weight (g)	134.33±5.20	135.33±6.67	139.33±4.8	145.62±5.21	146.00±4.04	154.33±2.6
Water intake (mL)	0	50.40±6.85	44.00±3.51	40.00±1.15	48.67±3.71	36.00±4.16
Food consumption (g)	0	24.33±2.40	24.67±1.67	24.00±1.53	28.30±0.86	24.33±1.20
Faeces output (g)	0	8.00±0.58	12.67±0.33	15.33±1.33	19.67±0.33	14.00±1.00
Urine output (mL)	0	15.53±4.78*	7.80±3.29	6.83±2.13	11.43±4.30*	7.50±3.12
Male (Vehicle 10 mL/kg)						
Body weight (g)	126.8±3.79	131.33±5.36	138.38±7.4	145.33± 4.96	145.33±5.78	151.67±6.1
Water intake (mL)	0	40.43±1.84	46.67±5.70	45.00±10.00	51.00±8.62	42.00±6.43
Food consumption (g)	0	33.33±1.33	23.33±1.86	24.38±2.67	26.67±2.33	22.33±3.38
Faeces output (g)	0	9,67±0.86	13.00±2.52	15.00±2.08	20.33±1.76	14.33±2.40
Urine output (mL)	0	5.90±1.34	5.43±1.79	3.60±1.40	3.87±0.33	3.77±0.22
Female (1000 mg/kg)						
Body weight (g)	132±0.58	133.67±5.40	131.00±8.0	135.33±9.83	133.33±6.36	132.00±4.1
Water intake (mL)	0	57.20±5.18*	42.67±1.67	42.67±3.71	39.00±1.53	31.33±1.33
Food consumption (g)	0	24.00±1.00	22.00±1.00	23.00±1.15	26.67±0.33	21.00±1.00
Faeces output (g)	0	10.00±1.15	12.03±1.20	16.33±1.20	21.20±2.93	11.33±0.88
Urine output (mL)	0	14.17±2.67	7.90±2.08	7.57±1.22	5.83±0.67	5.03±1.44
Female (Vehicle 10mL/kg)						
Body weight (g)	124.67±4.18	127.33±8.35	135.35±4.9	135.33±4.91	137.55±7.08	132.20±3.5
Water intake (mL)	0	42.93±2.95	45.00±0.58	45.00±0.51	52.67±2.19	40.00±1.15
Food consumption (g)	0	23.67±2.33	22.33±1.86	23.00±3.21	20.67±3.21	14.33±2.18
Faeces output (g)	0	8.33±1.33	12.00±1.00	16.67±2.33	20.67±3.48	14.33±2.19
Urine output (mL)	0	12.33±1.69	5.60±1.06	10.33±3.48	9.43±1.95	5.10±2.21

The values represent the mean ± SEM. One-way ANOVA, followed by the Student-Newman-Keuls test. *p <0.05 vs. vehicle.

Table 2: Effect of 28 days oral daily administration of CVO (1000 mg/kg, p.o.) on haematological parameters in male and female rats.

Parameter	Male (1000 mg/kg)	Treatment		
		Male (Control)	Female (1000 mg/kg)	Female (Control)
Red blood cells (10^{12}/L)	7.47±0.52	7.09±0.28	7.18±0.36	6.90±0.14
Hemoglobin (g/L)	149.67±10.65	142.67±4.81	146.00±7.09	138.33±3.53
Hematocrit (%)	39.50±2.79	37.74±1.56	38.85±2.22	37.05±1.34
MCV[a] (fL)	52.90±0.10	53.23±1.25	54.03±0.43	53.67±1.29
MCH[b] (Pg)	20.08±0.17	20.17±0.26	20.35±0.02	20.10±0.29
MCHC[c] (g/L)	379.00±2.65	378.33±4.91	376.33±3.38	374.00±4.00
Platelets (10^9/L)	393.33±13.96*	503.67±56.46	561.33±99.62	687.67±224.17
Total leukocytes (10^9/L)	2.27±0.50	2.57±0.64	3.53±0.53	3.70±0.55
Neutrophils: Relative (%)	10.38±1.59	11.07±1.42	14.07±3.35	12.54±2.31
Absolute (10^9/L)	0.25±0.07	0.30±0.11	0.53±0.18	0.47±0.10
Lymphocytes: Relative (%)	67.28±2.05	64.15±1.95	61.81±3.54	64.41±2.09
Absolute (10^9/L)	1.51±0.30	1.62±0.36	2.17±0.30	2.32±0.34
MXD[d] : Relative (%)	22.34±1.03	24.78±0.93	24.12±1.53	24.72±0.86
Absolute (10^9/L)	0.51±0.14	0.64±0.17	0.83±0.09	0.92±0.15

The values represent the mean ± SEM. One-way ANOVA, followed by Student-Newman-Keuls test. [a]MCV: mean corpuscular volume; [b]MCH: mean corpuscular hemoglobin; [c]MCHC: mean corpuscular hemoglobin concentration; [d]MXD: Monocytes–basophils–eosinophils mixed; *p < 0.05 vs control

Table 3: Effect of daily oral administration of CVO (1000 mg/kg, p.o.) for 28 days on biochemical parameters in male and female rats.

Parameter	Male (1000 mg/kg)	Treatment		
		Male (Control)	Female (1000 mg/kg)	Female (Control)
Glucose (mg/dL)	154.94±30.54	150.42±24.75	144.72±13.09	167.42±13.74
Urea (mg/dL)	64.53±5.44	59.98±7.87	54.50±3.46	49.02±0.59
Creatinine (mg/dL)	2.01±0.01	1.99±0.02	2.06±0.01	2.01±0.04
Uric acid (mg/dL)	4.81±0.18	4.28±0.47	3.60±0.55	4.41±0.64
Alanine amino transferase (UI/L)	22.73±3.47	25.47±9.56	9.20±2.91	8.07±1.99
Aspartate amino transferase (UI/L)	105.17±8.69	100.83±12.67	91.83±15.30	79.83±2.73
Alkaline phosphatase (UI/L)	718.06±93.41	711.62±65.34	876.30±197.26*	411.70±70.12
Total Cholesterol (mg/dL)	117.19±10.81	105.47±3.40	95.64±1.89	97.15±2.95
Triglycerides (mg/dL)	108.14±8.49	110.60±12.90	104.15±9.97	92.47±5.84
Total proteins (mg/dL)	6.69±0.75	7.39±0.19	7.22±0.35	7.51±0.33
Gamma-glutamyl transferase (UI/L)	5.02±0.39	2.70±0.39	1.93±0.77	3.09±1.02
Albumin (mg/dL)	4.37±0.43	4.64±0.07	4.81±0.02	5.01±0.06
D-bilirubin (mg/dL)	1.80±0.19	1.56±0.18	1.06±0.28	0.78±0.11
T-bilirubin (mg/dL)	1.23±0.13	1.08±0.10	0.83±0.23	0.60±0.13
High-density lipoproteins (mg/dL)	81.93±4.56	83.50±7.12	102.87±27.37	76.17±12.47
Chlorine (mg/L)	1145.22±28.37	1132.54±83.54	1305.80±180.53	1311.09±167.75
Potassium (mg/L)	223.51±41.30	241.51±39.67	173.00±3.71	176.49±24.09

The values represent the mean ± SEM. One-way ANOVA, followed by Student-Newman-Keuls test. *p < 0.05 vs control

Table 4: Effect of oral daily administration of CVO (1000 mg/kg, p.o.) for 28 days on relative weight of the internal organs of rats.

Organs weight index	**% of body weight**			
	Male (1000 mg/kg)	Male (Control)	Female (1000 mg/kg)	Female (Control)
Brain	1.49±0.10	1.27 ± 0.15	1.51±0.05	1.40±0.26
Liver	5.37±0.41	5.44 ± 0.46	4.67±0.15	4.81±0.32
Spleen	0.66±0.02	0.66± 0.06	0.48±0.04	0.61±0.09
Right kidney	0.40±0.06	0.57±0.03	0.49±0.10	0.42±0.02
Left kidney	0.49±0.08	0.50±0.02	0.58±0.17	0.40±0.01
Lung	1.22±0.06	1.22±0.23	1.07±0.17	0.90±0.06
Heart	0.54±0.07	0.51±0.02	0.44 ±0.05	0.42±0.01
Stomach	5.76±0.57	3.56±0.33	2.40±0.53	3.87±0.31
Testes	2.85±0.57	2.71±0.62		
Uterus			1.26±0.31	0.99±0.15

The values represent the mean ± SEM (n = 5)

Antitussive effect

The test product, CVO, exhibited dose-dependent suppression of cough bouts (Figure 1). The maximum dose tested produced an effect that was comparatively to that produced by the centrally acting cough-suppressant, dihydrocodeine. The effect produced by 200 and 800 mg/kg doses were statistically significant (p < 0.01) relative to the untreated control group.

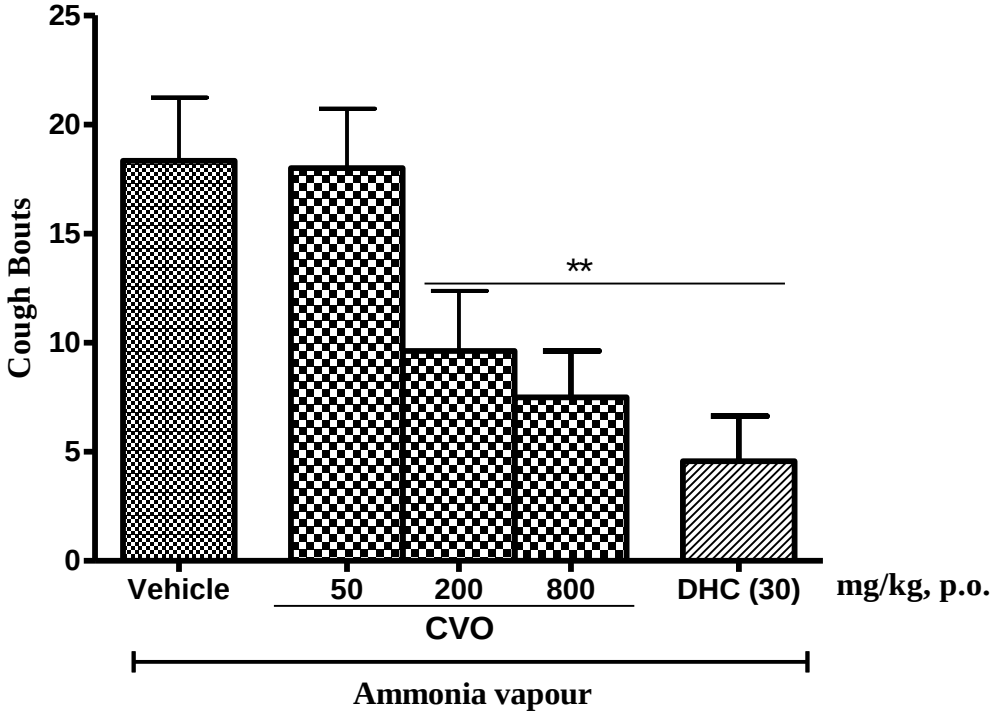

Figure 1: Antitussive activity of CVO in ammonia vapor-induced cough. Each column represents the mean ± S.E.M. of 6 animals. One-way ANOVA followed by Student-Newman-Keuls test. ** p <0.01 vs. vehicle.

Expectorant activity

This was determined by quantifying phenol red-stained mucus secreted in mouse trachea, using a standard calibration plot of phenol red. As shown in table 5, CVO produced slight increase in tracheal mucus expectoration, relative to the negative control. This effect super ceded the effect produced by the standard expectorant, ammonium chloride significantly. Phenol red standard calibration plot ($y = 0.1181x-0.02$ $R^2 = 0.9988$)

Table 5: Effect of CVO on tracheal mucus expectoration

Treatment	Dose (mg/kg)	Concentration (µg/ml)
Aqueous vehicle	10 ml/kg	0.5771 ± 0.075
CVO	50	0.5460 ± 0.081
	200	0.5378 ± 0.040
	800	1.734 ± 0.276**
Ammonium chloride	500	10.560 ± 1.900a***

Values are represented as mean ± standard error of mean (SEM), **$p < 0.01$, ***$p < 0.001$, (Student unpaired t-test); a= $p < 0.001$ (one way ANOVA)

Antipyretic activity

A typical biphasic febrile response with peaks between 1 and 3 h after LPS injection was observed, with maximum fever peak at 2 h after injection (Fig. 2). Prophylactic administration (1 h before LPS injection) of various doses (50 - 800 mg/kg) of CVO resulted in slight suppression of fever, particularly within the first three hours. The reduction in body temperature produced by 800 mg/kg CVO was significant (p <0.05) within 1.5 and 2 h. This dose, however, was not as effective as the positive control, indomethacin in sustaining the antipyretic effect for up to 3 h.

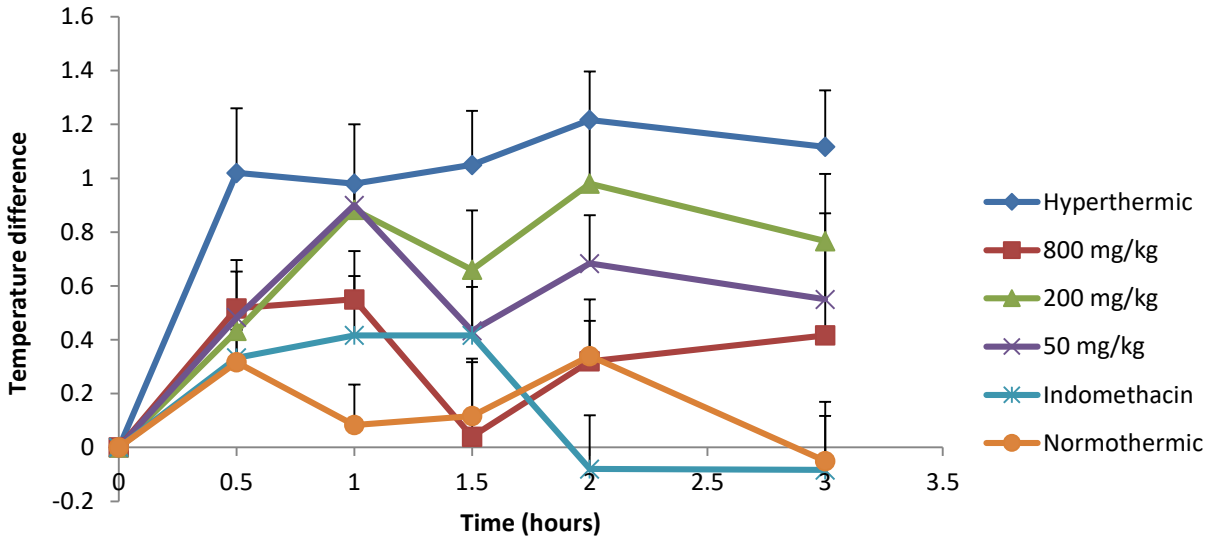

Figure 2: Effect of CVO treatment on LPS-induced febrile response in mice

It was observed that CVO dose-dependently reduced the general febrile response compared to the untreated hyperthermic group (Fig. 3). However only the standard drug, indomethacin, significantly ($p < 0.05$) suppressed the febrile response over time and temperatures within this group were comparable with that of the untreated, normothermic control.

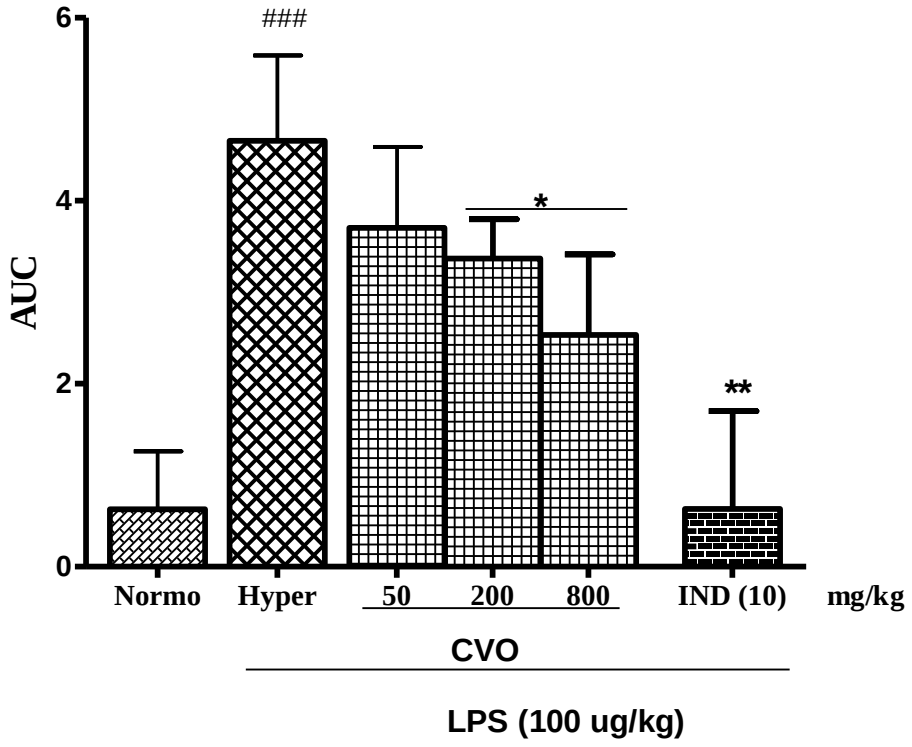

Figure 3: Effect of treatment of CVO on Temp. Difference-time response areas under curve (AUC). Each column represents the mean ± S.E.M. of 6 animals. One-way ANOVA followed by Student-Newman-Keuls test. *$p < 0.05$; and ** $p < 0.01$ vs. vehicle; $^{###}p < 0.001$ vs. sham.

Discussion

The physiochemical parameters of the infusion extract indicates that CVO contains 4.2 g (=> 70 mg/kg, in adult) of the active constituents, deduced from the percentage (%) yield. Extrapolating from the formula for dose translation based on body surface area; the limit test dose used in rats for this assay (2000 mg/kg) translate to over 400% of equivalent dose in adult human [10]. Safety of an agent can be established by assessing the acute toxicity using the limit test [4]. The test is used in situations where there is information indicating that the test material is likely to have toxicity below regulatory limit doses. No mortality was recorded in all the mice that received 2000 mg/kg, p.o. of CVO. According to the Globally Harmonized Classification (GHS) system, test agents that produce no mortality at doses of 2000-5000 mg/kg are considered as Category 5 ranges [11]. This indicates that it does not

possess the potential to be lethally injurious on acute oral consumption. The test substance CVO is therefore safe based on the model used. The model can predict toxicological effects, and by extrapolation [10] gives a high predictive value in humans [12]. It should be noted however that this study did not cover other route of administration, effects of long-term use, or organ histological evaluation of the test systems.

The sub-chronic test allow for the characterization of the toxicological profile of the test material on several parameters in target organs, as well as indication of no-observed-effect level (NOEL) and the no-observed-adverse-effect-level (NOAEL). These include: clinical signs and symptoms, body weight changes, water and feed intake, excretion of feces and urine, macroscopic and relative weight of the main organs and hematological and biochemical tests.

In the sub-chronic test, CVO did not alter any parameters related to weight gain, water consumption and excretion of feces and urine, except for the increase in urine output in the male group and water intake in the female group, which reversed after 2 days of the observation of the effect in both cases. This shows CVO does not alter the normal physiology of the animals.

A low platelet count as observed in the male group can occur even if the bone marrow makes enough platelets as the body could destroy its own platelets due to autoimmune responses. Despite the significant decrease in the platelets count, the value was still within the physiological ranges for Wistar rats [13]. Although increase in alkaline phosphatase observed in the female group may indicate a cause of concern, this event did not present in the macro organ analysis and the values are also within the physiological ranges for Wistar rats [13, 14].

The doses of CVO used for the efficacy assays were 50, 200, and 800 mg/kg. These doses were extrapolated using the formula for dose translation based on the conversion factor from human doses to that for rats and mice [15]. Cough is produced when sensory receptors in the airways are excited by mechanical and/or chemical stimuli [16]. The excitability of the sensory receptors themselves can be modified by drugs, and compounds that suppress cough by this mechanism are defined as peripheral antitussive agents. Airway sensory afferents control the excitability of neural elements in the brainstem that produce cough, and drugs that act at this level of the CNS, like dihydrocodeine, are classified as centrally acting antitussive agents. Cough-suppressant therapy commonly incorporates the use of pharmacologic agents with mucolytic effects and/or inhibitory effects on the cough reflex itself. This type of therapy intends to reduce the frequency and/or intensity of coughing, especially on a short-term basis. In the test model we employed, we measured the effect of CVO on cough frequency alone and it was found to be active in suppressing cough. To further characterize this product based on its effect on the respiratory tract, we also

investigated its effect on tracheal mucus expectoration.

Expectorants increase secretion of mucins and are defined as drugs that induce discharge or expulsion of mucus from the respiratory tract. The mucosecretory property of expectorants may be associated with mucolysis or a decrease in mucus viscosity, beta-2 adrenoceptor stimulation, or an increase in mucociliary clearance [17]. In this investigation, CVO produced a minimal expectorant effect, compared to ammonium chloride. Thus, CVO may be acting specifically to suppress the cough reflex, and not primarily through mucosecretory mechanisms.

Lipopolysacchariade is an essential component of the outer cell membrane of all gram-negative bacteria and is the most potent pyrogen associated with infections caused by gram-negative bacilli. Therefore, we evaluated the antipyretic effect of CVO in the LPS-induced rat pyrexia. In this model, LPS (100 µg/kg)-induced elevation in core body temperature in fasted rats peaks within 3 h and is spontaneously attenuated afterward [18]. Fever results from the endotoxin injection primarily due to prostaglandin E_2 release (among other inflammatory mediators like IL-1, TNF, endothelin), and is reversed by central and peripherally acting anti-inflammatory drugs. Indomethacin is a peripherally acting non-steroidal anti-inflammatory drug that blocks peripheral prostaglandin synthesis and action. The nature and plausible mechanism of the antipyretic effect of CVO needs to be evaluated further.

Conclusion

CVO is safe, can symptomatically reduce cough bouts and fever. It should however be noted that evaluating the claim of prevention or cure of COVID-19 infection by CVO is outside the scope of this study.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors appreciate Mr. Sunday Dzarma, Mr. Solomon Fidelis, and Mrs. Faustina Nwachukwu for their technical assistance.

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