Original Article

Acute and Subacute Oral Toxicity Characterization and Safety Assessment of COVID Organics® (Madagascar's Anti-COVID Herbal Tea) in Animal Models

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Abstract

Introduction: Coronavirus disease 2019 (COVID-19) is an infectious disease caused by severe acute respiratory syndrome coronavirus 2. No drug has been generally approved as safe and effective for the treatment of COVID-19. Several therapeutic agents such as COVID Organics® (CVO) have been explored as treatment options. CVO is an herbal tea composed of 62% of *Artemisia annua* and 38% of other plants. There is presently no existing scientific report and data on the safety and efficacy of CVO herbal drug. Thus, acute and subacute toxicity studies were undertaken to evaluate the safety and toxicity of CVO on short- and long-term usage in animal models. **Materials and Methods:** Phytochemical and nutritional compositions of CVO were determined using standard methods. Acute oral toxicity was investigated using female Swiss albino mice (three per group). While subacute oral toxicity was done using female and male Swiss albino rats (five per group). The animals were administered 2000 mg/kg, 5000 mg/kg, therapeutic dose; 5500 mg/kg and supratherapeutic dose; 11,000 mg/kg of CVO herbal product. The control group received water *ad libitum.* The oral toxicity studies were done in accordance with Organization for Economic Corporation and Development guidelines. The experimental protocol was approved by the Institutional Animal Care and Use Committee, Nigerian Institute of Medical Research (Ethics No. IRB/17/043). **Results:** CVO is rich in antioxidants: flavonoids (10.3%), tannins (29.1%), and phenolics (434.4 mg). It contains proteins (33.8%), carbohydrates (34.5%), fat (6.8%), and fiber (0.5%). In the acute toxicity study, no mortality was recorded in all the treated and untreated groups. The lethal dose of CVO is >5000 mg/kg body weight. The hematological, biochemical, lipid profile, and histologic parameters were all normal at therapeutic doses when compared to the control group. **Conclusion:** The acute and subacute oral toxicity studies revealed that CVO is not toxic. The specific organ toxicity evaluations also indicated that CVO has no toxic effects on blood parameters and vital organs structure and function at therapeutic dose. Thus, CVO is safe for short- and long-term usage. We recommend that CVO should be subjected to efficacy studies to investigate whether it is effective for COVID-19 treatment as claimed by the manufacturer.

Keywords: Coronavirus disease 2019, herbal medicines, histopathology, phytochemicals, proximate analyses, toxicity assessment

Résumé

Introduction: La maladie à coronavirus 2019 (COVID-19) est une maladie infectieuse causée par le coronavirus 2 du syndrome respiratoire aigu sévère. Aucun ne médicamenta été généralement approuvé comme étant sûr et efficace pour le traitement du COVID-19. Plusieurs agents thérapeutiques comme le COVID Organics® (CVO) ont été explorées comme options de traitement. CVO est une tisane composée à 62% d'Artemisia annua et à 38% d'autres plantes. Il y a actuellement il n'existe aucun rapport scientifique ni aucune donnée sur l'innocuité et l'efficacité du médicament à base de plantes CVO. Ainsi, des études de toxicité aiguë et

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subaiguë ont été entreprises évaluer la sécurité et la toxicité du CVO sur une utilisation à court et à long terme dans des modèles animaux. **Matériels et méthodes:** phytochimiques et les compositions nutritionnelles du CVO ont été déterminées à l'aide de méthodes standard. La toxicité orale aiguë a été étudiée chez des femmes albinos suisses souris (trois par groupe). La toxicité orale subaiguë a été réalisée sur des rats albinos suisses femelles et mâles (cinq par groupe). Les animaux étaient administrés 2 000 mg/kg, 5 000 mg/kg, 5 500 mg/kg (dose thérapeutique) et 11 000 mg/kg (dose suprathérapeutique) de produit à base de plantes CVO. Le le groupe témoin a reçu de l'eau à volonté. Les études de toxicité orale ont été réalisées conformément à l'Organisation pour la société économique et Directives de développement. Le protocole expérimental a été approuvé par le Comité institutionnel de protection et d'utilisation des animaux de l'Institut nigérian de Recherche médicale (Éthique n° IRB/17/043). **Résultats:** Le CVO est riche en antioxydants : flavonoïdes (10,3 %), tanins (29,1 %) et phénoliques (434,4 mg). Il contient des protéines (33,8 %), des glucides (34,5 %), des lipides (6,8 %) et des fibres (0,5 %). Dans l'étude de toxicité aiguë, aucune mortalité n'a été enregistrée chez tous les groupes traités et non traités. La dose mortelle de CVO est > 5 000 mg/kg de poids corporel. Le profil hématologique, biochimique, lipidique et les paramètres histologiques étaient tous normaux aux doses thérapeutiques par rapport au groupe témoin. **Conclusion:** Les conséquences orales aiguës et subaiguës des études de toxicité ont révélé que le CVO n'est pas toxique. Les évaluations de la toxicité spécifique pour certains organes ont également indiqué que le CVO n'a aucun effet toxique sur le sang. Paramètres et structure et fonction des organes vitaux à dose thérapeutique. Ainsi, CVO est sans danger pour une utilisation à court et à long terme. Nous recommandons que Le CVO doit être soumis à des études d'efficacité pour déterminer s'il est efficace pour le traitement du COVID-19, comme le prétend le fabricant.

Mots-clés: Maladie à coronavirus 2019, plantes médicinales, histopathologie, produits phytochimiques, analyses immédiates, évaluation de la toxicité

Introduction

The emergence of coronavirus disease 2019 (COVID-19) has caused thousands of deaths and great economic recession ever witnessed in decades.[1,2] As of November 22, 2020, over 57.8 million confirmed COVID-19 cases and 1.3 million deaths have been reported to the World Health Organization from across the world; out of these statistics, Africa has 1.4 million confirmed cases and 24, 464 deaths and new more cases and mortality are still recorded on daily basis.[1] There is currently a serious search for a safe and effective therapeutics and vaccines to cure infected persons and prevent the transmission of COVID-19 worldwide.[3]

COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[4] SARS-CoV-2 has a spherical structure enveloped by spike proteins with which the virus assembles and attaches itself to a host cell angiotensin converting enzyme 2 receptors for effective invasion.[5] It attacks mostly the upper and lower respiratory system of its host causing severe acute respiratory syndrome and other symptoms such as fever, dry cough, shortness of breath, sore throat, and other constitutional symptoms like hyperimmunologic responses (cytokine storm), respiratory failure, and eventually death in severe cases but can present little or no symptoms in some infected persons (asymptomatic cases).[3]

Since the outbreak of SARS-CoV-2, no drug has been generally approved for the treatment of the infectious disease.[6] Various countries resorted to the use remdesivir, Traditional Chinese Medicine, hydroxychloroquine sulfate (Plaquenil), chloroquine phosphate, lopinavir/ritonavir, convalescence plasma or serum, arbidol, anticytokinic biologic agents (anakinra or tocilizumab), COVID organics® (CVO), monoclonal antibody (Immunoglobulin G1), mesenchymal stem cells, and certain dietary supplements.[6-8]

CVO is an herbal tea made up of 62% *Artemisia annua* and 38% of unnamed Madagascar's plants. CVO was endorsed by the Madagascar's President as a cure for COVID-19 and subsequently distributed it to many African countries.[9] Nigeria received the package, but our President emphasized that the safety of the herbal tea must be ascertained by evidence-based scientific safety evaluations before its use on COVID-19 patients.[10] Even though previous studies on Artemisia plant revealed that it is safe, the adverse effect that may arise from the synergistic reaction of *A. annua* and the other Madagascar's plants is unknown. Herbal medicines can sometimes be toxic due to synergistic reactions of the many phytonutrients within, or from contamination, lack of standardization, or errors during plant harvest.[11,12] Hence, it has been emphasized that medicinal plants should be subjected to safety and efficacy evaluations before clinical use to protect human health and prevent dangerous health hazards.[13,14] There is presently no existing scientific report on the safety and efficacy of CVO herbal drug. Given the widespread distribution and use of CVO along with the lack of information on its safety and toxicity, These acute and subacute oral toxicity studies were undertaken to evaluate the safety and toxicity potential of CVO on hematological, biochemical, and histological parameters upon short- and long-term consumption in mammals.

Materials and Methods

Herbal tea preparation

CVO herbal tea was prepared according to the manufacturer's description. Briefly, 2.85 g of the herbal tea was brewed in 125 mL of boiled water for 15 min to extract the bioactive compounds after which it was filtered. The filtrate was collected, allowed to cool, and administered to the animals in 24 h. This was repeatedly prepared through the 28-day study depending on the volume required daily.

Product description

- Product name: CVO Tambavy Tisane herbal tea.
- Manufacturer: Institut Malagasy des Recherches Appliquees (Madagascar)
- Indications: Strengthens the immune system, treats viral infections, reduces fever, and cures breathing difficulties
- Dosage: Adults are to drink 33 cl twice per day, while children are to drink 33 cl per day.

Acute oral toxicity study

A total of 15 female Swiss albino mice (3 animals per group) that weighted 20.45–21.63 g ± 20% were used. They were nulliparous and nonpregnant. The animals were obtained from the institutional animal house, Department of Biochemistry and Nutrition, Nigerian Institute of Medical Research (NIMR). They acclimatized for 5 days to laboratory condition. The mice were maintained at a room temperature of 25°C ± 3°C and a 12 h light/dark cycle. The female mice were given standard animal pellets (Ladokun Feeds, Ibadan) and tap water *ad libitum.*

The acute oral toxicity was investigated according to the guidelines for acute oral toxicity study of the Organization for Economic Corporation and Development (OECD) no. 423.[15] The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) (Ethics No. IRB/17/043). Four different groups consisting of four fixed doses namely 2000 mg/kg, 5000 mg/kg, 5500 mg/kg (therapeutic dose recommended by the manufacturer for humans) and 11,000 mg/kg (supratherapeutic dose that is double of the recommended dose) of CVO were used and the untreated group served as the control. The animals were observed for signs of toxicity and mortality for 14 days.

Phytochemical analysis

Phytochemical analysis was carried out on CVO herbal tea to identify the active phytochemical constituents both qualitatively and quantitatively. The phytochemical screenings were performed on the herbal preparation using standard procedures described elsewhere.[16,17]

Proximate analysis

Proximate analyses were carried out on CVO to determine its nutritional compositions using conventional method.[18]

Subacute oral toxicity study

The subacute-oral toxicity test was done using female and male Swiss albino rats with weight of 124–160 g. They were obtained from the institutional animal house. The subacute toxicity was done in accordance with OECD guidelines for repeated dose 28-day oral toxicity test.[19] The experimental protocol was approved by the IACUC, Department of Biochemistry and Nutrition, NIMR (Ethics No. IRB/17/043). Three fixed doses, namely subtherapeutic dose (2750 mg/kg), therapeutic dose (5500 mg/kg) and supratherapeutic dose (11,000 mg/kg) were used. Animals were administered daily with the different doses of the herbal extracts for a period of 28 days.

Hematological analysis

At the end of the acute and subacute toxicity studies, blood samples were collected from the ocular sinus of the mice and rats into anticoagulant tubes for analysis of the effect of CVO on these hematological parameters: white blood cells (WBCs), red blood cells (RBCs), hemoglobin (HGB), hematocrit (HCT), mean corpuscular hemoglobin (MCH), MCH concentration, and platelets using automated hematology analyzer.

Clinical chemistry analysis

Blood samples of the animals in each group of the acute and subacute toxicity studies were collected into a plain bottle on day 14 and day 29, respectively, and sera were obtained by centrifuging the blood using an electrical centrifuge. The sera were used for blood clinical biochemistry analysis (glucose, urea, creatinine, total protein, alanine transaminase, aspartate

Table 1: Hematological parameters report

Hematological parameters	Control	Subtherapeutic dose (5000 mg/kg)	Therapeutic dose (5500 mg/kg)	Supratherapeutic dose (11,000 mg/kg)	Treated groups, mean±SD	*P*
WBC (×10^9/L)	6.1	5.0	7.5	14.0	8.83±4.64	0.42
HGB (g/dL)	8.1	13.9	14.3	12.1	13.43±1.17	0.01*
RBC (×10^{12}/L)	5.32	8.30	8.28	7.22	7.93±0.64	0.02*
HCT (%)	24.5	42.4	42.7	36.8	40.63±0.29	0.04*
MCV (fL)	46.2	51.1	51.6	51.1	51.27±0.29	0.01*
MCH (pg)	15.2	16.7	17.2	16.7	16.87±0.29	0.01*
MCHC (g/L)	33.0	32.7	33.4	32.8	32.97±0.38	0.89
RDW-CV (%)	17.8	15.4	14.6	16.6	15.53±1.01	0.06
RDW-SD (fL)	27.8	27.0	27.0	28.6	27.53±0.92	0.67
PLT (×109/L)	237	991	953	1013	985.67±30.35	0.01*
MPV (fL)	6.8	7.4	7.7	6.9	7.33±0.40	0.15
PDW (fL)	14.0	15.7	16.2	14.5	15.47±0.87	0.10

P value marked with asterisk (*) in [Table 1] were significant when compared to the control value. *P* value was set to be significant when <0.05 (*P*<0.05). SD=Standard deviation, WBC=White blood cell, HGB=Hemoglobin, RBC=Red blood cells, HCT=Hematocrit, MCH=Mean corpuscular hemoglobin, MCHC=MCH concentration, RDW=Red cell distribution width, CV=Coefficient of variation, PLT=Platelet, MPV=Mean platelet volume, PDW=Platelet distribution width, MCV=Mean corpuscular volume

transaminase, and lipid profile) to test renal and hepatic functions using Automated Clinical Chemistry Analyzer.

Histopathological analysis

The animals were sacrificed by cervical dislocation after the acute and 28-day studies following blood collection. Vital organs (liver, kidneys, lungs, and heart) were removed through a midline incision in the rat's abdomen. The organs were placed in 10% formalin containing tubes before been subjected to histopathological examination. Features of cell injury, cellular vacuolization, pyknosis, hemorrhage, necrosis, inflammation, glomerular filtrations, fibrosis, vascular changes, fatty deposits, and focal lesions were investigated.

Statistical analysis

The data were analyzed using one sample *t*-test by comparing the values of the animals that received various doses of CVO and the control group using STATA statistical software version 13.0 (STATA Corp., College station, Texas, USA). Significant level was set at $P<0.05$. The data were presented as mean ± standard deviation.

Results

Acute systemic toxic effects

In this study, no death, morbidity, and clinical signs of toxicity such as diarrhea, salivation, coma, tremor, skin damage, blindness, and hair loss was observed during the 14-day study. The lethal dose (LD_{50}) was estimated to be above 5000 mg/kg body weight.

Acute effect of COVID Organics® on hematological parameters

The result of hematological parameters evaluated is shown in Table 1. The hematological parameters result of the treated group were normal when compared with control group values. The WBC count of the animals that received therapeutic and supratherapeutic doses of CVO had higher WBC counts than the control group. Furthermore, the RBCs, HGB, and HCT levels of the animals treated with the herbal tea were statistically significantly increased at the treatment doses.

Acute effect of COVID Organics® on serum biochemical parameters

The results of the biochemical analysis are shown in Table 2. The results revealed that the liver and kidney function parameters of the animals that received the therapeutic dose of CVO were normal when compared to the control. Similarly, the blood glucose, lipid level, and total protein level were also not statistically higher than the control group.

Acute effects of COVID Organics® on vital organs histology

Acute effect on the heart

The results of the histopathological analysis of heart organs excised from the animals treated with CVO and the untreated group (control) are shown in Figure 1, which includes photomicrograph a and b. These results showed no abnormal histopathological effects on the hearts of the animals that were treated with CVO even at the supratherapeutic dose compared to the cardiac structure of the untreated group.

Acute effect on the kidney

The results of the histopathological analysis of kidney organs excised from the animals treated with CVO and the untreated group (control) are shown in Figure 1, which includes photomicrograph c and d. Figure 2 shows some histopathological alterations (glomerular sclerosis) in the kidney of treated and untreated groups. The safety of CVO on kidney histology was validated in a repeated-dose toxicity study (subacute oral toxicity testing).

Acute effect on the liver

The results of the histopathological analysis of liver organs excised from the animals treated with CVO and the untreated group (control) are shown in Figure 1, which includes

Table 2: Serum biochemical parameters

Serum biochemistry parameters	Control	Subtherapeutic dose (5000 mg/kg)	Therapeutic dose (5500 mg/kg)	Supratherapeutic dose (11,000 mg/kg)	Treated groups, mean±SD	*P*
ALT (U/L)	67.3	60.4	41.9	87.2	63.17±22.78	0.78
AST (U/L)	134.9	150.8	134.2	273.8	189.27±73.30	0.33
ALP (U/L)	165	241	152	233	208.67±49.24	0.26
BILD (mmol/L)	0.7	0.9	0.5	0.9	0.77±0.23	0.67
BILT (mmol/L)	1.1	2.0	1.3	2.4	1.9±0.56	0.13
Creatinine (µmol/L)	20 L	22 L	23 L	19 L	21.33±2.08	0.38
Urea (mmol/L)	12.1	10.0	8.5	10.2	9.56±0.93	0.04*
Albumin (g/L)	32.8	30.2	29.2	31.1	30.17±0.95	0.04*
Total protein (g/L)	62.3	59.1	60.2	60.9	60.07±0.91	0.05
Glucose (mmol/L)	6.83	9.03	7.05	6.15	7.41±1.47	0.57
TRIG (mmol/L)	1.59	1.29	1.35	1.61	1.42±0.17	0.22
LDL (mmol/L)	0.41	0.53	0.38	0.54	0.48±0.09	0.29
HDL (mmol/L)	1.99	2.33	1.97	2.28	2.19±0.20	0.21
Total cholesterol (mmol/L)	2.30	2.65	2.32	2.58	2.52±0.17	0.16

P values marked with asterisk (*) in [Table 1] were significant when compared to the control value. *P* value was set to be significant when <0.05 ($P<0.05$). SD=Standard deviation, ALT=Alanine transaminase, AST=Aspartate transaminase, ALP=Alkaline phosphatase, HDL=High-density lipoprotein, LDL=Low-density lipoprotein, BILD=Direct bilirubin, BILT=Total bilirubin, TRIG=Triglyceride

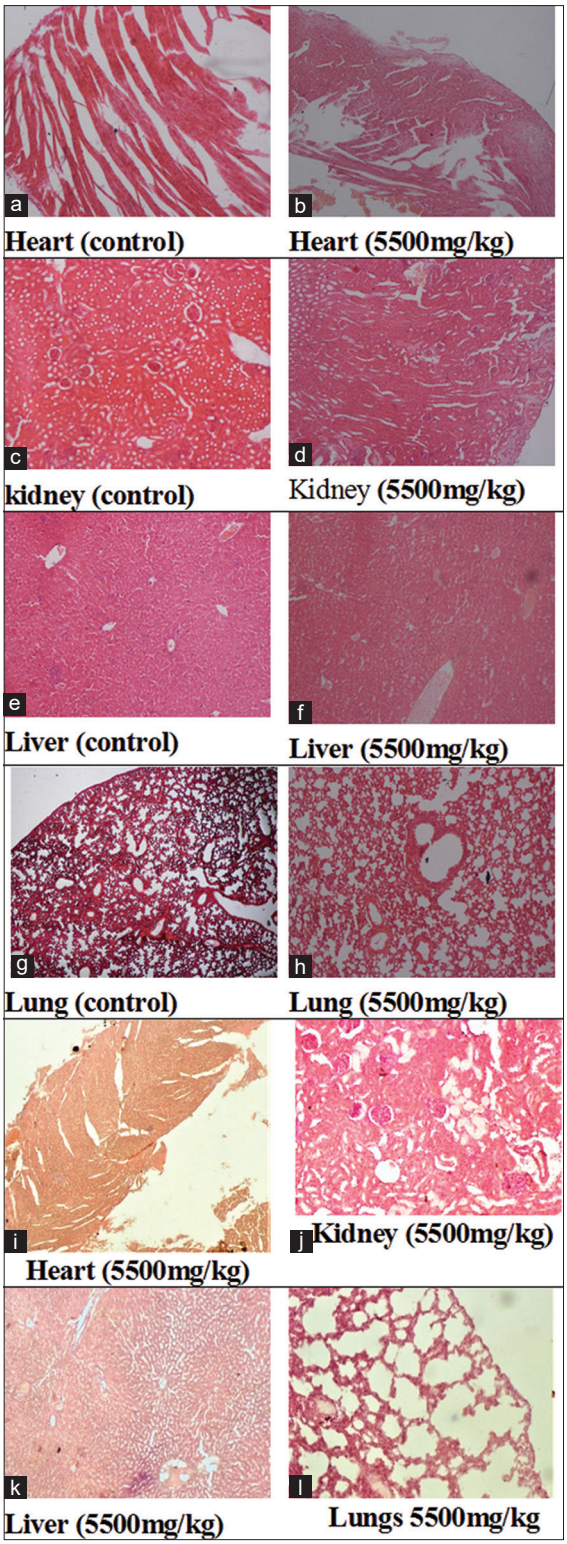

Figure 1: Acute and subacute toxicity histopathology photomicrographs: Histologic sections of heart, kidney, liver, and lungs, (a) normal heart, (b) normal heart, (c) mild interstitial nephritis seen, (d) global glomerular sclerosis seen, (e) mild portal triaditis was seen, (f) normal liver, (g) moderate congestion seen, (h) moderate interstitial pneumonia Histologic sections of heart, kidney, liver and lungs for subacute toxicity, (i) normal heart, (j) normal kidney, (k) normal liver, (l) mild edema in lung

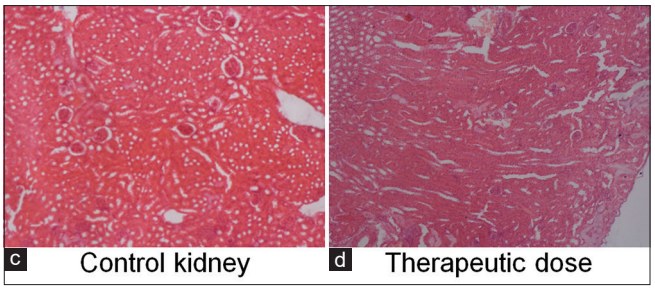

Figure 2: Histologic sections of kidney tissue show cellular glomerular tufts disposed on a background containing renal tubules. (c) Mild interstitial nephritis seen. (d) Global glomerular sclerosis (probably long standing) seen

Table 3: Qualitative analysis report for COVID organics® phytochemical constituents

Active phytochemicals	Inference
Flavonoids	+
Terpernoids	+
Cardiac glycosides	+
Tannins	+
Phlobatanin	+
Steroid	−
Saponin	+
Alkaloids	+
Phenol	+
Anthraquinone	+

+=Positive, −=Negative, ®=Product trade mark

photomicrograph e and f. Figure 3 reveals normal histology of the liver at therapeutic dose but shows mild hepatic injury at overdose.

Acute effect on the lung

The results of the histopathological analysis of lungs excised from the animals treated with CVO and the untreated group are shown in Figure 1, which includes photomicrograph g and h. Furthermore, Figure 4 shows mild interstitial pneumonia in both treated and untreated groups when subtherapeutic and therapeutic doses were used. However, no histological abnormalities were seen in the lungs of the animals who received the supratherapeutic dose of CVO.

Phytochemical analysis result

The results of the phytochemical analysis carried out on CVO are presented in Tables 3 and 4.

Proximate analysis report

The proximate analysis revealed the presence of the following nutritional components listed in Table 5.

Subacute toxic effects

In the female rats used for this 28-day toxicity study, no treatment related death, and morbidity were observed for all female rats that were treated with CVO both at the therapeutic dose and at overdose. No clinical signs of toxicity such as diarrhea, salivation, coma, tremor, skin damage, blindness,

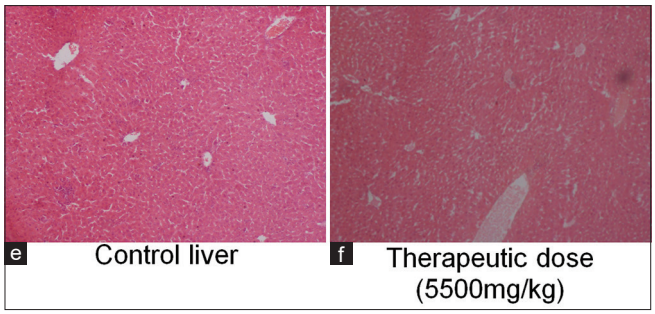

Figure 3: Histologic sections of liver show general structure, central vein (CV), portal vein (PV) and the basophilic portion with nucleus and the acidophilic cytoplasm of the acinar cells. (e) Mild portal triaditis was seen. (f) Normal liver

Table 4: Quantitative analysis report for COVID organics® phytochemical constituents

Active phytochemicals	Percentage
Flavonoids	10.3
Tannins	29.07
Saponin	33.7
Alkaloids	1.2
Phenol	434.4 mg GAE/100 g

GAE=Gallic acid equivalent, ®=Product trade mark

Table 5: Proximate analysis report

Parameters	Percentage
Moisture content	8.4
Crude fat	6.8
Crude protein	33.8
Crude fiber	0.5
Ash content	16
Carbohydrates	34.5

and hair loss was observed during the 28-day study. However, three deaths were recorded in the male rats during the course of the study. These occurred in clinical dosage group (1 death), supratherapeutic dosage group (1 death), and the control group (1 death). The specific cause of these death is unknown. The hematological, biochemical, and histopathological examinations may provide further details regarding the safety and toxic characteristics of the test substance.

Subacute effect of COVID Organics® on vital organs histology

The results of the histology analysis of vital organs (kidney, liver, lungs and heart) excised from both treated and untreated groups of the female and male rats are shown in Figures 1 from i to l.

Effects on the heart

The results of the histology analysis of the heart are shown in Figure 1, which includes photomicrograph i. The histologic sections of the heart revealed no cardiac injuries like myocarditis, pericarditis, and atrophy in the control and treatment group at the therapeutic dose. However, consistent heart injuries were seen in the overdose group of female and male animals.

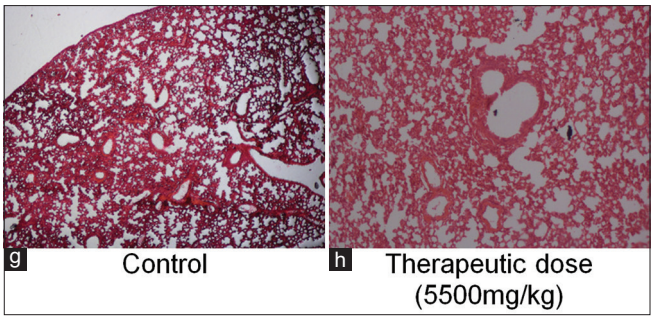

Figure 4: Histologic sections of lung tissue show alveolar air spaces and bronchioles on a background interstitium containing blood vessels. (g) Moderate interstitial pneumonia, moderate oedema and congestion seen (h) Moderate interstitial pneumonia, moderate oedema and congestion seen

Effect on the kidney

The results of the histology analysis of the kidneys are shown in Figure 1, which includes photomicrograph j. In this repeated dose study (28 days), the histologic sections revealed no form of renal damage such as acute congestion, acute tubular necrosis, interstitial nephritis, and glomerular sclerosis in all the groups of the treated animals at both therapeutic dose and overdose. This was also the same for the untreated groups.

Effect on the liver

The results of the histology analysis of the kidneys are shown in Figure 1, which includes photomicrograph k. There was no remarkable alterations in the morphology of the liver cells in the control and treated animals of both sexes.

Effect on the lungs

The results of the histology analysis of the kidneys are shown in Figure 1, which includes photomicrograph l. The histopathological examination of lung tissues showed no pulmonary injury at the therapeutic dose. But at supratherapeutic doses there was evidence of edema and lung congestion. However, the untreated group also showed mild alterations in the morphology of the lungs.

Discussion

Phytochemical and proximate analysis

The presence of flavonoids and tannins in CVO indicates that it may have antioxidant activities because flavonoids and tannins are known antioxidants in plants.[20] It may also have antiviral activity because of the good amount of phenols, tannins, and saponins present in the herbal tea. Similarly, the result of the proximate analysis indicates that CVO contains more of proteins and carbohydrates and less fats and oil.

Acute and subacute general toxicity

The safety of herbal medicines are evaluated via acute toxicity, subacute toxicity, subchronic toxicity, chronic toxicity, multigeneration toxicity, reproductive toxicity, and carcinogenicity studies and/or genotoxicity studies.[13,21]

The absence of death in the acute toxicity study, morbidity, and clinical signs of toxicity during the 14 days study indicates that CVO is nontoxic or lethal at the therapeutic dose even at the supratherapeutic dose (overdose). The LD_{50} was above 5000 mg/kg body weight. A substance with an LD_{50} > 2000 mg/kg body weight is considered to be safe.[22]

Hematological and biochemical effects

Hematological parameters such as HCT, HGB, and numbers of erythrocytes and WBCs provide information of toxicity on blood elements, while biochemical parameters are often used as markers of hepatic and renal functions and/or impairment.[23,24] The acute toxicity hematology result indicates that CVO is safe on blood of the animals up to the highest dose (supratherapeutic dose). It also suggests that CVO may have immune boosting activities and antiviral and/or antibacterial effects due to the significant increase in the WBCs which help the body to fight foreign pathogens. The statistically significant increases in the RBCs, HGB, and HCT levels in the treated groups indicate that CVO stimulates the production of erythrocytes. Furthermore, the lack of statistically significant increase in the clinical biochemistry parameters of the treated animals when compared to the controls shows that CVO is safe on hepatic and renal functions, blood glucose, and lipid levels at therapeutic dose.

Acute and subacute histopathological effects

Histopathological assessment is an essential safety assessment technique that have long been used for investigation of possible pathological alterations induced in laboratory animals by an external substance.[25] The five most essential targets of toxicity are kidney, liver, brain, lungs, and heart.[26] In this studies, histopathological analysis was conducted on four of these vital organs in order to evaluate any toxic effects that CVO herbal remedy may have on them.

Effect on heart histology

The lack of abnormal histopathological effects on the hearts of the animals that were treated with CVO even at the supratherapeutic dose (overdose) compared to the untreated group during the acute toxicity study indicates that CVO has no adverse effect on cardiac histology. Hence, the herbal tea is safe on the heart at therapeutic dose and higher dose upon short- and long-term consumption. The consistent heart injuries seen in the overdose group of female and male animals suggests that CVO could cause mild degree of damage to the heart at overdose.

Effect on kidney histology

The presence of glomerulosclerosis in the kidney sections of treated and untreated animals in the acute toxicity is unclear. Glomerulosclerosis is a marker of kidney damage which could be caused by host inherent diseases or external factors like drugs/chemicals. The absence of renal damage such as acute congestion, acute tubular necrosis, interstitial nephritis, and glomerular sclerosis in all the groups of the treated animals at both therapeutic dose and overdose indicates that CVO herbal tea is not toxic to the kidney at long-term usage.

Effect on liver histology

The absence of histopathology in the liver of the animals during acute toxicity indicates that CVO is safe on the liver at the recommended dose. The subacute toxicity study also revealed no remarkable alterations in the structure and morphology of the liver sections in the control and treated animals of both sexes. The liver is the main organ involved in the biotransformation and detoxification of xenobiotics and metabolic end products in biological systems. The absence of hepatotoxicity shows that CVO is safe on the liver even at long-term consumption.

Effect on lung histology

The presence of lung injury in the acute toxicity may have been caused by injury sustained during the oral administration. In the subacute toxicity study, no pulmonary injury was seen at the therapeutic dose but at supratherapeutic doses there were evidence of edema and lung congestion. However, the untreated group also showed mild alterations in the histology of the lungs. This may mean that the changes were not induced by the CVO. Hence, CVO is safe on the lungs.

Conclusion

The acute and subacute oral toxicity studies revealed that CVO is not toxic. The specific organ toxicity evaluations also indicated that it has no toxic effects on blood parameters and vital organs morphology, structure, and function at therapeutic dose. Thus, CVO is safe for short- and long-term usage. CVO may possess antioxidant and antiviral activities because of its high content of antioxidants especially tannins and saponins. We recommend that CVO should be subjected to efficacy studies to investigate whether it is effective for COVID-19 treatment as claimed by the manufacturer.

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Conflicts of interest

There are no conflicts of interest.

References

1. World Health Organization. WHO Corona Virus Disease (COVID-19) Dashboard: World Health Organization; 2020. Available from: https://covid19.who.int/table?tableDay=yesterday. [Last accessed on 2020 Nov 22].
2. World Bank. The Global Economic Outlook during the COVID-19 Pandemic: A Changed World; 2020. Available from: https://www.worldbank.org/en/news/feature/2020/06/08/the-global-economic-outlook-during-the-covid-19- pandemic-a-changed-world. [Last accessed on 2020 Nov 22].
3. Baden LR, Rubin EJ. COVID-19 – The search for effective therapy. N Engl J Med 2020;382:1851-2.
4. Gorbalenya AE, Baker SC, Baric R, Groot RJ, Drosten C, Gulyaeva AA, *et al.* Severe acute respiratory syndrome-related coronavirus: The species and its viruses – A statement of the coronavirus study group. Nat Microbiol 2020;5:536-44.
5. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, *et al.* SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. Cell 2020;16;181(2):271-280.e8.

6. McKee DL, Sternberg A, Stange U, Laufer S, Naujokat C. Candidate drugs against SARS-CoV-2 and COVID-19. Pharmacol Res 2020;157:104859.
7. Shoenfeld Y. Corona (COVID-19) time musings: Our involvement in COVID-19 pathogenesis, diagnosis, treatment and vaccine planning. Autoimmun Rev 2020;19:102538.
8. Yang Y, Xiao Z, Ye K, He X, Sun B, Qin Z, *et al.* SARS-CoV-2: Characteristics and current advances in research. Virol J 2020;17:117.
9. Felix T. WHO to Study Madagascar's Drug to Treat COVID-19 Anadolu Agency; 2020. Available from: https://www.aa.com.tr/en/africa/who-to-study-madagascars-drug-to-treat-covid-19-/1840971. [Last accessed on 2020 Aug 14, Last updated on 2020 May 14].
10. Punch Nigeria. UPDATED: Buhari Receives Madagascar's COVID Organics Nigeria: The Punch News Paper; 2020. Available from: https://punchng.com/updated-buhari-receives-madagascars-covid-organics/. [Last accessed on 2020 Aug 17, Last updated on 2020 May 16].
11. El-Nahhal Y. Contamination and safety status of plant food in Arab countries. Contamination and safety status of plant food in Arab countries. J Appl Sci 2004;4:411-7.
12. National Toxicology Program. Toxicology and carcinogenesis studies of *Ginkgo biloba* extract (CAS No. 90045-36-6) in F344/N Rats and B6C3F1/N mice (gavage studies). Natl Toxicol Program Tech Rep Ser 2013;(578):1-183.
13. Ekor M. The growing use of herbal medicines: Issues relating to adverse reactions and challenges in monitoring safety. Front Pharmacol 2014;4:177.
14. World Health Organization. WHO Traditional Medicine Strategy: 2014-2023. Geneva: World Health Organization; 2013.
15. OECD. Test No. 423: Acute Oral Toxicity – Acute Toxic Class Method. Paris: OECD; 2002.
16. Harborne JB. A Guide to Modern Techniques of Plant Analysis. Springer Dordrecht: Chapman and Hall; 1973.
17. Sofowora A. Medicinal Plants and Traditional Medicinal in Africa. 2nd ed. Sunshine House, Ibadan, Nigeria. Spectrum Books Limited: Screening Plants for Bioactive Agents; 1993.
18. Oyagbemi AA, Odetola AA, Azeez OI. Phytochemical investigation and proximate analysis on the leaves of *Cnidoscolus aconitifolius*. J Med Food 2011;14:322-4.
19. OECD. Test No. 407: Repeated Dose 28-day Oral Toxicity Study in Rodents. Paris:OECD; 2008.
20. Sankhalkar S. Antioxidant enzyme activity, phenolics and flavonoid content in vegetative and reproductive parts of *Moringa oleifera* Lam. Am J Pharm Res 2014;4:255-70.
21. World Health Organization. Guidelines for the Assessment of Herbal Medicines. Geneva: World Health Organization; 1991.
22. OECD. Test No. 423: Acute Oral toxicity - Acute Toxic Class Method [Internet]. Paris: Organisation for Economic Co-operation and Development; 2002 [cited 2023 Oct 17]. Available from: https://www.oecd-ilibrary.org/environment/test-no-423-acute-oral-toxicity-acute-toxic-class-method_9789264071001-en.
23. Denny KH, Stewart CW. Acute, Subacute, Subchronic, and chronic general toxicity testing for preclinical drug development. In: Faqi AS, editor. A Comprehensive Guide to Toxicology in Nonclinical Drug Development. 2nd ed., Ch. 5. Boston: Academic Press; 2017. p. 109-27.
24. OECD. Test No. 412: Subacute Inhalation Toxicity: 28-Day Study. Paris: OECD; 2018.
25. Greaves P. Histopathology of Preclinical Toxicity Studies: Interpretation and Relevance in Drug Safety Evaluation. Academic Press; 2011.
26. Chisvert A, Salvador A. Analysis of Cosmetic Products. Amsterdam: Elsevier; 2007.