Anti-nociceptive and antipyretic activities of *CoV-Pla 3* extract: relevance on symptomatic treatment of Covid-19 Disease

Bukata B. Bukar[1, 2], Kakjing D. Falang[1, 2,], Kennedy I. Amagon[1, 2], Yusuf A. Agabi[1, 3], Richard J. Kutshik[1, 4], Ajima Ukpe[1, 5], Yohanna L. Ishaya[1, 4], Simji S. Gomerep[1, 6], Stephen D. Davou[1, 7], Ismaila Shittu[1, 8], James G. Damen[1, 9], Noel N. Wannang[1, 2], Jacob A. Kolawole[1, 5]

[1]Plateau State Research Committee on Covid-19 and Other Infectious Diseases, Government House, Rayfield, Jos.
[2]Department of Pharmacology and Toxicology, University of Jos, Jos
[3]Virology Unit, Department of Microbiology, University of Jos, Jos
[4]Department of Biochemistry, University of Jos, Jos
[5]Department of Pharmaceutical and Medicinal Chemistry, University of Jos, Jos
[6]Infectious Disease Unit, Department of Medicine, University of Jos
[7]Plateau State Ministry of Health, Jos
[8]National Veterinary Research Institute (NVRI), Vom, Plateau state
[9,]Department of Medical Laboratory Sciences, University of Jos, Jos

Corresponding author: Bukata B. Bukar
Email: bukata33@yahoo.com, Phone: +2348036183979

ABSTRACT

Background: There is an urgent need to search for effective remedies against COVID-19 from the rich and extensive flora of Africa.

Objectives: The aim of this study was to evaluate the anti-nociceptive and anti-pyretic activities of the methanol extract of a herbal combination product, *Cov-Pla3* in experimental animals.

Methods: Animal models of nociception and hyperthermia including acetic acid-induced contortion, hot-plate-induced nociception, egg albumin-induced paw edema and Brewer's yeast-induce hyperthermia were adopted.

Results: The results revealed that the *Cov-Pla3* extract at all the doses used increased the reaction time in a non-significant manner ($p > 0.05$). However, the increase in reaction time at doses of the extract was significant compared to the baseline ($p < 0.05$). The results also showed that *Cov-Pla3* extract at the dose of 125 mg/kg at 90 minutes after administration had the highest anti-nociceptive effect of 49.73 % followed by 46.70 % at the dose of 500 mg/kg at same period.
It was also shown that the extract significantly reduced acetic acid-contortions at a dose of 500 mg/kg ($p < 0.05$) with corresponding inhibition of 73 %. The extract at all the doses used significantly decreased paw edema compared to baseline after 180 minutes of administration ($p < 0.05$). The anti-pyretic test indicated that *Cov-Pla3* significantly reduced temperature at 125 mg and 500 mg/kg after 4 hours of administration ($p < 0.05$). In conclusion, *Cov-Pla3* possesses a combination of analgesic, anti-inflammatory and antipyretic activities.

Conclusion: This scientific evidence, in addition to its safety profile from the LD_{50} of above 5000 mg/kg, justifies its recommendation for clinical trial use in Covid-19 patients with severe symptoms.

Keywords: Phytotherapy, Covid-19, Nociception, Hyperthermia, *Cov-Pla3*, Coronavirus

Activités anti-nociceptives et antipyrétiques de l'extrait de *CoV-Pla 3* : pertinence sur le traitement symptomatique de la maladie Covid-19

Bukata B. Bukar[1, 2], Kakjing D. Falang[1, 2], Kennedy I. Amagon[1, 2,] Yusuf A. Agabi[1, 3,] Richard J. Kutshik[1, 4,] Ajima Ukpe[1, 5], Yohanna L. Ishaya[1, 4], Simji S. Gomerep[1, 6], Stephen D. Davou[1, 7], Ismaila Shittu[1, 8], James G. Damen[1, 9], Noel N. Wannang[1, 2], Jacob A. Kolawole[1,5]

1Comité de recherche de l'État de Plateau sur le Covid-19 et autres maladies infectieuses, Government House, Rayfield, Jos.
2Département de pharmacologie et de toxicologie, Université de Jos, Jos
3Unité de virologie, Département de virologie, Université de Jos, Jos
4Département de biochimie, Université de Jos, Jos
5Département de chimie pharmaceutique et médicinale, Université de Jos, Jos
6Unité des maladies infectieuses, Département de médecine, Université de Jos
7Plateau Ministère de la santé de l'État de Plateau, Jos
8Institut national de recherche vétérinaire (NVRI), Vom, État de Plateau
9Département des sciences de laboratoire médical, Université de Jos, Jos

Auteur de correspondance: Bukata B. Bukar
Email: bukata33@yahoo.com, Téléphone: +2348036183979

RÉSUMÉ

Contexte : Il est urgent de rechercher des remèdes efficaces contre le COVID-19 dans la flore riche et vaste de l'Afrique.

Objectifs : Le but de cette étude est d'évaluer les activités anti-nociceptives et antipyrétiques de l'extrait au méthanol d'un produit combiné à base de plantes, *Cov-Pla3* en essai de laboratoire sur des animaux.

Méthodes : Des modèles animaux de nociception et d'hyperthermie, y compris la contorsion induite par l'acide acétique, la nociception induite par la plaque chauffante, l'œdème des pattes induit par l'albumine d'œuf et l'hyperthermie induite par la levure de bière ont été adoptés.

Résultats : Les résultats ont révélé que l'extrait de *Cov-Pla3* à toutes les doses utilisées a augmenté le temps de réaction de manière non significative ($p > 0,05$). Cependant, l'augmentation du temps de réaction aux doses de l'extrait était significative par rapport à la valeur initiale de référence ($p <0,05$). Les résultats ont également montré que l'extrait de *Cov-Pla3* à la dose de 125 mg/kg à 90 minutes après l'administration a eu l'effet anti-nociceptif le plus élevé de 49,73% suivi de 46,70% à la dose de 500 mg/kg à la même période.
Il a également été démontré que l'extrait a réduit de manière significative les contorsions d'acide acétique à une dose de 500 mg/kg ($p <0,05$) avec une inhibition correspondante de 73%. L'extrait à toutes les doses utilisées a réduit de manière significative l'œdème de la patte par rapport à la valeur initiale de référence après 180 minutes d'administration ($p <0,05$). Le test antipyrétique a indiqué que *Cov-Pla3* a significativement réduit la température à 125 mg et 500 mg/kg après 4 heures d'administration ($p< 0,05$). En conclusion, *Cov-Pla3* possède une combinaison d'activités analgésiques, anti-inflammatoires et antipyrétiques.

Conclusion : Cette preuve scientifique, en plus de son profil d'innocuité à partir de la LD50 supérieure à 5000 mg/kg, justifie sa recommandation pour une utilisation d'essai clinique chez les patients de Covid-19 présentant des symptômes graves.

Mots-clés : Phytothérapie, Covid-19, nociception, hyperthermie, *Cov-Pla3*, Coronavirus

INTRODUCTION

Covid-19 disease is an infectious disease caused by a single-stranded virus called severe acute respiratory syndrome coronavirus type-2 (SARS-CoV2). It belongs to the Coronaviridae family together with other members that include Severe Acute Respiratory Syndrome Coronavirus (SARS-CoV) and Middle East Respiratory Syndrome Coronavirus (MERS-CoV). As of 17th July, 2020, more than 13.8 million cases were reported from among 188 countries with more than 590,000 deaths.[1] This figures increased to 22.9 million cases and 800, 321 deaths by 22nd August, 2020.[2] As of 22nd August, 2020, Plateau state was reported as having 2,113 confirmed cases with 29 deaths and 1,063 recovered, leaving a balance of 1,021 confirmed cases.[2]

Since the emergence of the first case of Covid-19 in Wuhan, China, in November, 2019[3] there have been several therapeutic attempts to treat the disease,[4,5] but most of it have been only symptomatic and supportive, whether the case is mild or severe.[6] In spite of these attempts, there still exist therapeutic gaps with all the agents that have been tried, of which the efficacy of most of the agents remain controversial. However, this is by no means a loss of hope as many agents both as orthodox and herbal formulations are still being investigated for possible effective treatment of the infection. Though improving the outcomes of Covid-19 patients with conventional antiviral such as remdesivir, lopinavir, oseltamivir and antibacterial such as azithromycin or amoxicillin have been reported, the outcomes remain unsatisfactory. There is currently no agreement on any claim of an effective therapy with any agent. This is largely due to the fact that no clinical trial has been conclusively performed on the efficacy of existing medicines on Covid-19. This therefore calls for more pro-active plans and strategies in tackling the disease.

It is on the basis of the above background that the Plateau State Research Team on Covid-19 and Other Infectious Diseases has come up with herbal formulations code-named *CoV-Pla1, CoV-Pla2 and CoV-Pla3.* These formulations are combination products of not less than five medicinal plants each known to be traditionally used in viral and bacterial infections among traditional communities in Nigeria and Africa at large. The individual medicinal plants have been investigated for some of their pharmacological properties with the results showing promising antiviral and immunomodulatory effects.

CoV-Pla3 is intended for use in patients with symptomatic and severe cases. The combination is in line with the use of herbal combinations to treat illnesses which is a common practice in many communities in developing countries. This is mainly for reason of enhanced therapeutic outcomes and benefits. This strategy is similar to therapy with orthodox drugs where more than one drugs are prescribed for the therapeutic management of an illness. It is with this approach in mind that *CoV-Pla3* was formulated with five different herbs in order to circumvent the possible mechanisms by which SARS-CoV2 attacks the body systems.

The aim of the present investigation was to establish the safety profile and some pharmacological properties of *CoV-Pla3* in experimental animals.

MATERIALS AND METHODS

Animals

Albino rats (139-194 g) and mice (20.0-37.5 g) of both sexes were employed for the purpose of this study. The animals were purchased from the Animal Experimental Unit, Department of Pharmacology and Toxicology of the University of Jos, Jos. They were approved and certified for the experiment by the Committee on protocol for use of experimental animals of the Department of Pharmacology and Toxicology, University of Jos, under the ethical certificate number F17.00379 issued to the Plateau Research Team on Covid-19 and Other Infectious Diseases dated 5th June, 2020. They were separated from the others and placed in stainless cages. They were fed with standard pellets purchased from a reputable dealer in Jos and water ad libitum before the start of the experiment. The animals were exposed to natural light and darkness at normal temperatures and allowed to acclimatize before the commencement of the experiments.

Drugs and Chemicals

Normal saline, 0.9 % w/v, Morphine (Antigen Ltd), Acetic acid 0.6 % (M & B), Brewer's yeast (ICH), Acetylsalicylic acid (Sigma), Diclofenac (Sigma) and raw Eggs.

Collection of Plant Materials

Five medicinal plants were collected from different locations in Pankshin and Jos North LGCs, Plateau state from reputable herbalists. Each plant was identified and authenticated by Mr Christopher John at the Federal School of Forestry, Jos, Plateau state, and a voucher

specimen FHJ/20/183-7 prepared and kept for each in their herbarium.

Preparation of Cov-Pla3 Extract

The leaves of the five medicinal plants were separately washed, dried under the shade and powdered. The powders were weighed in a ratio of 40:15:20:20:5 based on their determined potencies and combined as formulation to form *Cov-Pla3*. 1000 g of the *Cov-Pla3* powder was weighed and extracted continuously with 200 ml of methanol (70 %) in a soxhlet extractor (Dionex, US) for 72 hours. The extract was thereafter evaporated to dryness in a vacuum evaporator at 50^{0}C until a constant weight was achieved. The yield was calculated to be 13.8 %. The extract was then stored and preserved in a refrigerator at a controlled temperature of -5 ^{0}C until the commencement of the experiment. The doses of the extract used for the experiment were determined from an earlier pilot using the acute toxicity test (LD_{50}) results in mice.

$$\text{Yield (\%)} = \frac{\text{Weight of Extract}}{\text{Weight of Powder}} \times 100$$

(A) Test for Anti-nociceptive Activities

(i) Hot-Plate Test in Mice

25 albino mice of both sexes were randomly selected and divided into 5 groups of 5 animals each. They were then separately placed on a hot-plate analgesiometer (Orchid, India) adjusted at a temperature of 50.0 ± 0.5 ºC as previously described.[7] Reaction time to licking of the hind paw or attempt of jumping off the plate of 5 minutes before and 30, 60 and 90 minutes after oral administration of the extract at 125; 250 and 500 mg were recorded for each group. Morphine was administered by the intra-peritoneal (IP) route at a dose of 1 mg/kg (morphine is known to increase reaction time at the dose range of 1-10 mg/kg) while normal saline was administered in control group at a dose of 1 ml/100 g also by IP route. A cut-off time of 60 seconds was adopted to avoid any tissue damage.

(ii) Acetic Acid Test in Mice

The method described by Koster[8] was used for the acetic acid test. The mice were divided into five groups of six animals each. The extract was then administered by oral gavage in different doses of 125; 250 and 500 mg to animals in respective groups. Morphine was administered by the IP route at a dose of 1 mg/kg and normal saline at a dose of 1 ml/100 g by same IP route. The animals were then allowed to stay for 30 minutes after which acetic acid was administered by IP route at a dose of 0.1 ml/10 g. Thereafter, abdominal contortions (writhes) were carefully observed and scored within a period of 20 minutes. Animals in control group were administered normal saline 1 ml/100 g before the administration of acetic acid.

(iii) The Egg Albumin-Induced Paw Test

The method described by Niemeger[9] was adopted. 25 albino wistar rats of both sexes were divided into 5 groups of 5 rats each. The control group was administered normal saline 1 ml/100 g while the treated groups where administered the extract at doses of 125; 250 and 500 mg/kg respectively. The second group was administered diclofenac at a dose of 20 mg/kg. Animals were then allowed to stand for 30 minutes. Thereafter, 0.1 ml/100 g of a freshly egg albumin was administered into the sub-plantar region of the hind paw of each animal. The paw diameter was measured with the aid of a Vernier caliper at different time intervals after the injection of the egg albumin.

(B) Test for hypothermic activity

(i) Brewer's Yeast-induced Hyperthermia Test

The method described by Williamson[10] was adopted for this test. 50 albino wistar rats of both sexes were screened by initially determining their normal rectal temperatures by means of a clinical thermometer carefully inserted into cavity for a period of two minutes. Induction of pyrexia was then carried out by sub-cutaneous (SC) of 1 mg/kg of 20 % suspension of Brewer's yeast to each animal. The animals were then starved for 18 hours after which their rectal temperatures were determined. The first 25 of them found to have increased temperature of 1 ^{0}C and above were selected and divided

into 5 groups for the experiment. The first group was administered normal saline 1 ml/kg and the second group was administered acetylsalicylic acid 16 mg/kg (IP). The last three groups received 125, 250 and 500 mg/kg of the extract by oral gavage. Their temperatures were monitored and recorded after 0.5; 1; 2; 3; 4 and 5 hours after administration of extract, aspirin or normal saline. The rectal temperature of the test animals was measured.

Statistical Analyses

All results were expressed as mean ± SEM. They were subjected to statistical analyses using the two-way ANOVA on IBM SPSS statistic-23 tool at P = 0.05 significant level.

RESULTS

Table 1: Acute Toxicity Testing (LD_{50} Determination) of *Cov-Pla3*

Dose (mg/kg)	No. of Animals	Mortality
Phase 1		
10	3	0
100	3	0
1000	3	0
Phase 2		
1600	1	0
2900	1	0
5000	1	0

Effect of Cov-Pla3 extract on reaction time of mice

The effect of *Cov-Pla3* extract on reaction time using the hot-plate model of nociception are shown on table 1 and figure 1. The results revealed that the extract at all the doses used increased the reaction time ($p < 0.05$). However, the increase in reaction time at doses of the extract was significant compared to the baseline ($p < 0.05$), suggesting the increase could be time-dependent (Table 1). The results also showed that *Cov-Pla3* extract at the dose of 125 mg/kg at 90 minutes after administration had the highest anti-nociceptive effect of 49.73 % followed by 46.70 % at the dose of 500 mg/kg at same period. However, the effects at both doses were lower than that due to morphine (79.95 %) (Figure 1).

Table 2: Effect of Cov-Pla3 extract on reaction time (sec) in mice

Treatment	Reaction Time (sec)			
	-5	30	60	90
Control, N/saline 1ml/100g	7.64 ± 0.55	7.72 ± 0.66	8.38 ± 0.68	7.28 ± 1.04
Morphine, 1mg/kg	6.66 ± 0.77	14.40 ± 4.00*	17.72 ± 5.59*	13.10 ± 1.76*
Cov-Pla3, 125 mg/kg	6.82 ± 0.48	10.50 ± 1.37	9.38 ± 0.56*	10.90 ± 0.74*
Cov-Pla3, 250 mg/kg	6.16 ± 1.05	8.76 ± 1.62	8.40 ± 0.89	9.68 ± 0.75*
Cov-Pla3, 500 mg/kg	5.40 ± 0.43	9.80 ± 1.33*	10.36 ± 0.67*	10.63 ± 0.74*

*** = $p < 0.05$ compared to baseline (-5)**
N = 5
Note: Both morphine and Cov-Pla3 did not significantly increase reaction time at all doses used ($p < 0.05$)

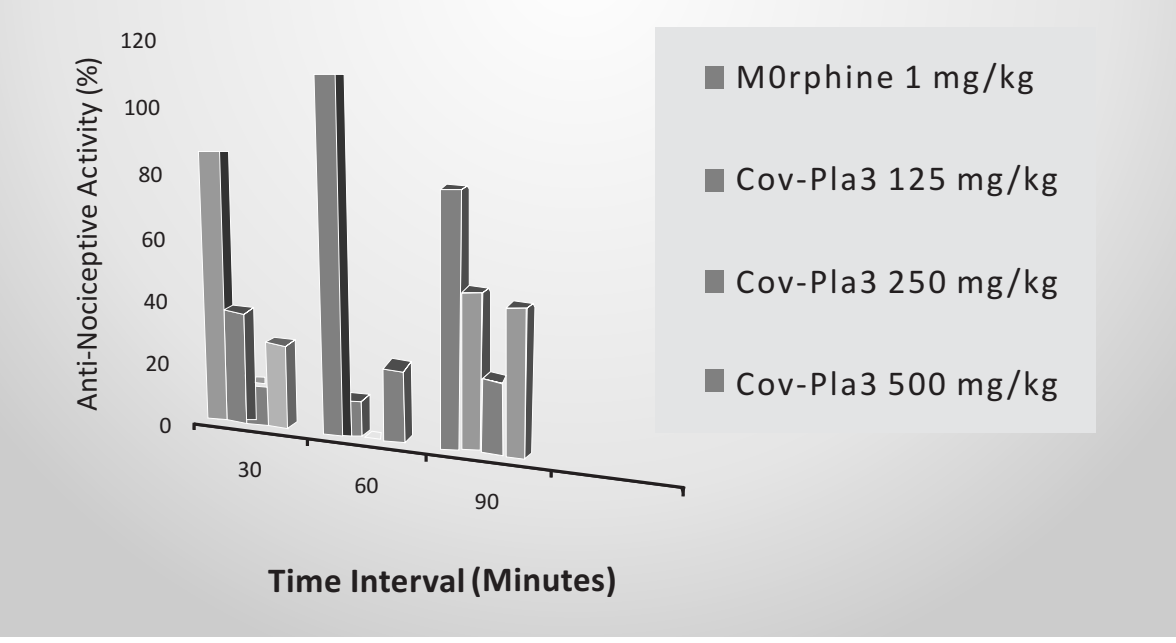

Figure 1: Analgesic effect of *Cov-Pla3* extract on hot plate induced nociception

Effect on acetic acid-induced contortion

The effect of *Cov-Pla3* effect on acetic acid-induced pain is presented on table 2. The results showed that the extract significantly reduced contortions at a dose of 500 mg/kg ($p < 0.05$) with corresponding inhibition of 73 %.

Table 3: Inhibitory effect of *Cov-Pla3* extracton acetic acid-induced pain in mice

Treatment	Contortion/20 Minutes	% Inhibition
Control, N/Saline, 1 ml/100 g	43.00 ± 8.51	-
Morphine, 1 mg/kg	4.80 ± 3.09*	88.84
Cov-Pla3, 125 mg/kg	36.40 ± 9.44	15.35
Cov-Pla3, 250 mg/kg	25.80 ± 9.96	40.00
Cov-Pla3, 500 mg/kg	11.40 ± 5.70*	73.49

*** = p < 0.05 compared to control group**
N = 6

Effect on egg albumin-induced inflammation

The effects of *Cov-Pla3* extract on egg albumin-induced paw edema are shown on table 3 and figure 2. The results showed that Cov-Pla3 extract exhibited a non-significant but time-dependent decrease of edema. The extract at all the doses used significantly decreased paw edema compared to baseline after 180 minutes of administration ($p < 0.05$). Similarly the extract at the dose of 500 mg/kg significantly reduced paw edema at all the time intervals compared to control ($p < 0.05$). Further analyses revealed that the inhibitory effect of the extract at 500 mg/kg was higher than that due to diclofenac 20 mg/kg at all the time intervals (Figure 2)

Table 4: Inhibitory effect of *Cov-Pla3* extract on egg albumin-induced paw edema (mm) in rats. Values are mean ± SEM

Treatment	Paw Edema (mm)/Time (sec)						
	Baseline	30	60	90	120	150	180
Control	4.15 ± 0.22	7.93 ± 0.24	8.09 ± 0.27	7.88 ± 0.22	7.57 ± 0.32	7.14 ± 0.21	6.93 ± 0.24
Diclofenac 20 mg/kg	4.34 ± 0.10	7.52 ± 0.07	7.52 ± 0.12	6.90 ± 0.18*	6.66 ± 0.06*	6.40 ± 0.15*	6.13 ± 0.07*a
Cov-Pla3 125 mg/kg	4.07 ± 0.06	7.44 ± 0.22	7.84 ± 0.20	7.78 ± 0.17	7.42 ± 0.29	6.63 ± 0.37	6.40 ± 0.27a
Cov-Pla3 250 mg/kg	3.94 ± 0.05	7.54 ± 0.35	7.74 ± 0.37	7.36 ± 0.35	6.78 ± 0.27	6.53 ± 0.34	6.34 ± 0.25a
Cov-Pla3 500 mg/kg	4.04 ± 0.04	6.82 ± 0.28*	6.89 ± 0.26*	6.71 ± 0.34*	6.51 ± 0.32*	6.09 ± 0.36*	5.91 ± 0.34*a

*** = p < 0.05 compared to control**
a = p < 0.05 compared to baseline
***a = both time and dose variations have significant effect**
N = 5

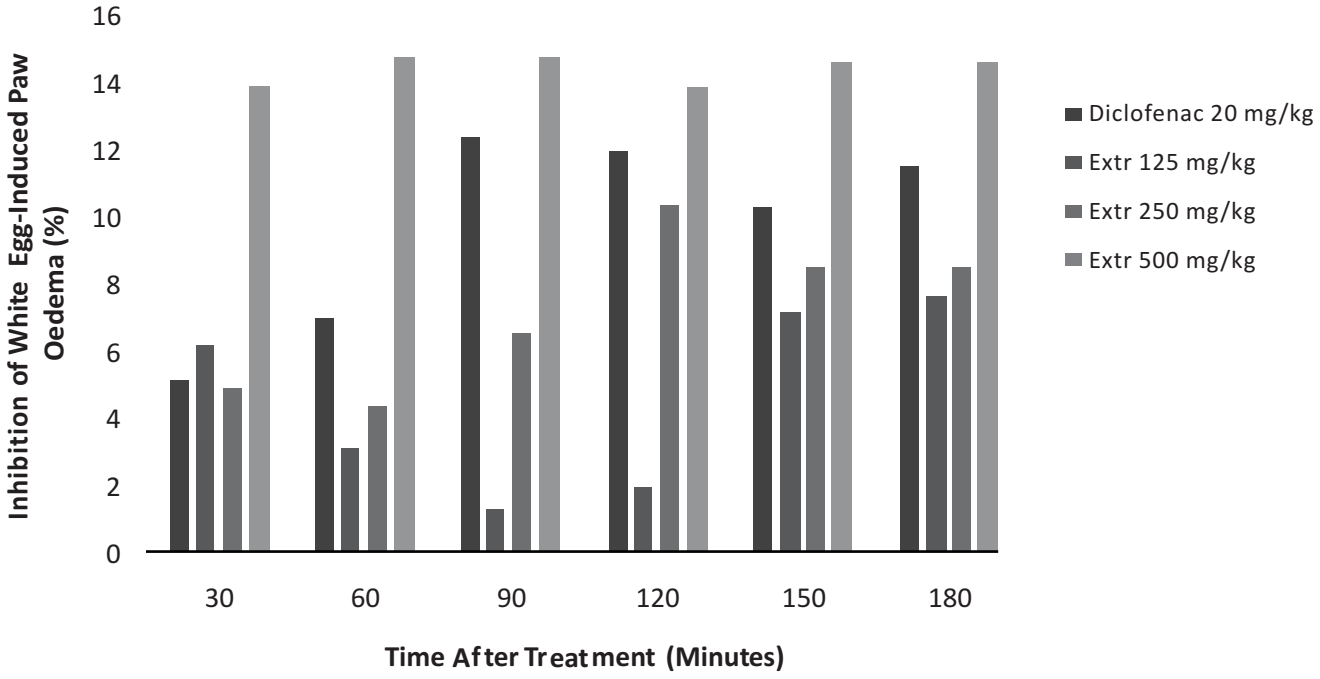

Figure 2: Inhibitory effect (%) of *Cov-Pla3* extract on egg albumin-induced paw edema in rats. Values are relative to control

Effect on Brewer's-induced hyperthermia

The results for the effect of *Cov-Pla3* on Brewer's-induced hyperthermia is shown on figure 3. The results indicate that Cov-Pla3 significantly reduced temperature at 125 mg and 500 mg/kg after 4 hours of administration ($p < 0.05$). However, the extract at all doses did not significantly reduce the temperature at all time intervals compared to control group ($p > 0.05$).

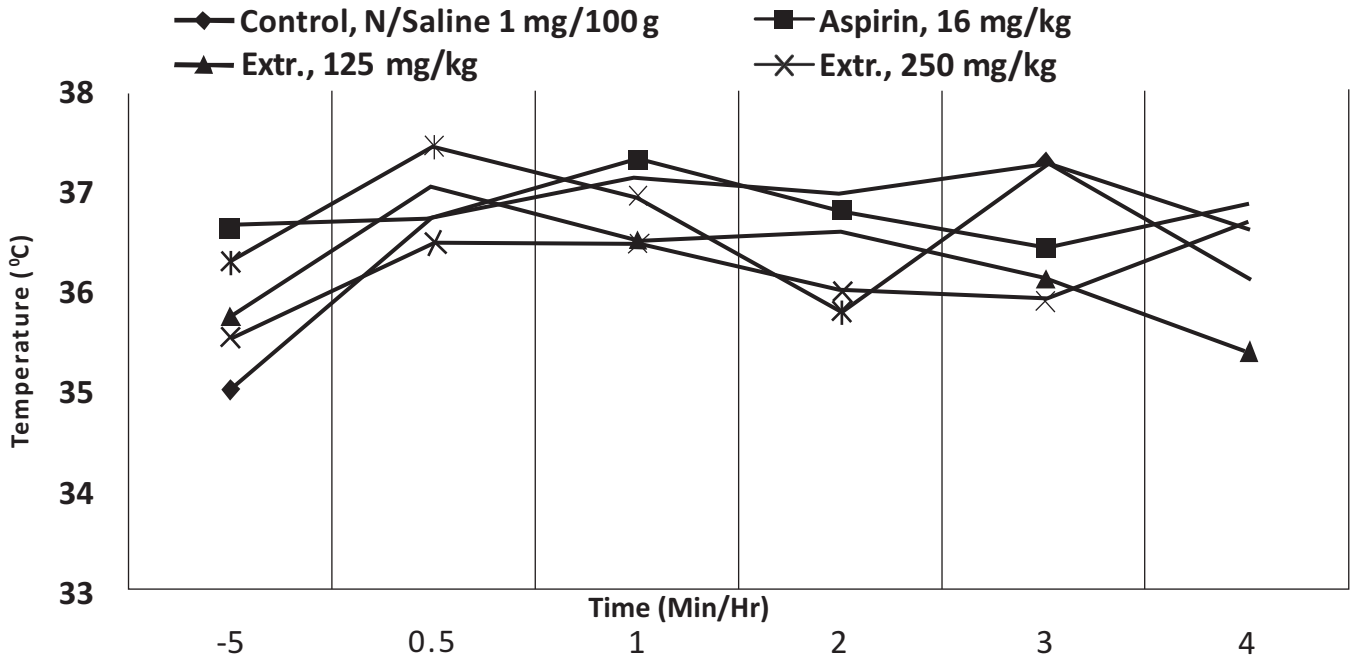

Figure 3: Antipyretic effect of *Cov-Pla3* Extract on Brewer's yeast-induced hyperthermia in rats

DISCUSSION

The anti-nociceptive and anti-pyretic effects of *Cov-Pla3* methanol extract were determined in experimental animals using standard animal models of nociception.

The hot-plate test is essentially a method for screening of centrally mediated nociception, but is also responsive to analgesics such as acetylsalicylic acid when the jumping reaction is considered.[11,12] The results revealed that *Cov-Pla3* was able to significantly increase reaction time thereby suggesting that *Cov-Pla3* possesses a central analgesic activity. Similarly, the acetic acid induced pain is known to be due to both central and peripheral mechanism, thereby equally suggesting that anti-nociceptive effect of *Cov-Pla3* may also be due to peripheral mechanism since all analgesic agents inhibit abdominal cramp. Taken together, these results suggest that the increase reaction could be due to increase in pain threshold mediated both centrally and peripherally.

The egg albumin test is similar to the carrageenan test and is a typical model for investigation of NSAIDs in acute inflammation.[13] The edema is an inflammatory response due to release of inflammatory mediators.[14] The results showed that *Cov-Pla3* extract exhibited a non-significant but time-dependent decrease of edema. The extract at all the doses used significantly decreased paw edema compared to baseline after 180 minutes of administration ($p < 0.05$). Similarly the extract at the dose of 500 mg/kg significantly reduced paw edema at all the time intervals compared to control ($p < 0.05$). These therefore suggest that *Cov-Pla3* may possess active principles that have anti-inflammatory activities.

The antipyretic test revealed that though, *Cov-Pla3* extract exhibited antipyretic activity, this may not be dose-dependent as the activity was shown to be significant only with time factor [($F(5, 72) = 3.38$, ($p < 0.05$)], suggesting a less frequent administration. It is reported that controlling high temperature may alleviate dangerous inflammatory responses and improve treatment outcome.[15,16]

Cov-Pla3 was formulated for treatment of Covid-19 patients with severe symptoms. Covid-19 is a disease that imposes a multi-systemic failure through different pathological mechanisms. Two of such identified mechanisms are inflammation and hyperthermia both of which manifest as fever. Indeed, fever, body pain and headache are some of the Covid-19 symptoms which manifest as increased nociception (pain sensation). Systemic inflammation in Covid-19 is associated with about 32.5 % of mortality.[17] In particular, fever remains a common symptom in majority of Covid-19 patients while elevated temperature correlates with the degree of inflammation and pain among such patients. Progression of the Covid-19 disease has been associated with immune failure due to exacerbation of the inflammatory components against the protective response mechanisms.[18] The use of immunosuppressive agents such as corticosteroid to manage hyper-inflammation in Covid-19 patients with severe symptoms has remained controversial among clinicians managing the disease. As a result there are evidences that suggest the non-steroidal anti-inflammatory drugs (NSAIDs) could be more effective for the management of inflammation in those with severe symptoms.[19]

In conclusion, *Cov-Pla3* possesses a combination of analgesic, anti-inflammatory and antipyretic activities. This scientific evidence, in addition to its safety profile from the LD_{50} of above 5000 mg/kg, justifies its recommendation for clinical trial use in Covid-19 patients with severe symptoms.

ACKNOWLEDGMENT

We wish to express our gratitude to Luka Wazoh, Bulus Diyen, Azi Sunday, Madaki Hoelleng Joshua, Joy Muplang Alexander, all of the Department of Pharmacology for their technical assistance and care for the animals. We are also indebted to BBB for the statistical analyses of results and typesetting the manuscript.

REFERENCES

1. Covid-19 Dashboard by the Center for Systemic Science and Engineering (CSSE) at Johns Hopkin's University. Available at https://coronavirus.jhu.edu/map.html. Accessed 17th July, 2020.
2. European Center for Disease Prevention and Control (ECDC). Updates on Covid-19. Available at https://www.ecdc.europa.eu/en/geographical-distribution-2019-ncov-cases. Accessed 22nd August, 2020.
3. Hui, D.S., Azhar, E.I., Madani, T.A., Ntoumu, F., Kock, R., Dar, O., *et al* (2020). The continuing 2019-nCov epidemic threat of novel coronavirus to global health-The latest 2019 novel coronavirus outbreak in Wuhan, China. *International Journal of Infectious Diseases* 91:264-266.
4. Youfifard, M., Zali, A., Ali, M.K., Neishaboori, A.M., Zarghi, A., Hosseini, M., and Safari, S. (2020). Antiviral therapy in management of covid-19: a systemic review on current evidences. *Archives of Academic Emergency Medicine* 8(1):e45.
5. Rismanbaf, A. (2020). Potential treatment for covid-19: a literature review. *Archives of Academic Emergency Medicine* 8(1):e29.
6. Wang, Y., Wang, Y., Chen, Y., and Qin, Q. (2020). Unique epidemiological and clinical features of the emerging 2019 novel coronavirus pneumonia (Covid-19) implicate special control measures. *Journal of Medical Virology* 92(6):568-576.
7. Jacob, J.J., and Ramabadran, K. (1978). Enhancement of nociceptive reaction by opiate antagonists in mice. *British Journal of Pharmacology* 64:91-98.
8. Koster, R.M., Anderson, M., and Beer, E.J. (1959). Acetic acid for analgesic screening. *Federation Proceedings* 18:412-416.
9. Niemeger, C.J., Verbiugen, F.J., and Janssen, P.A. (1964). Effects of various drugs on carrageenan-induced edema in rat hind paw. *Journal of Pharmacy and Pharmacology* 16:310-816.
10. Williamson, E.M., Okpako, D.T., and Evans, F.J. (1996). Pharmacological methods in phytotherapy research: selection, preparation and pharmacological evaluations of plant materials. *John Wiley and Sons,* NY, p147.
11. Carter, R.B. (1991). Differentiating analgesic and non-analgesic drug activities on rat hot plate: effect of behavioral end point. *Pain* 47:211-220.
12. Le Bars G., Gozariu, M., and Cadden, S.W. (2001). *Animal models of nociception. Pharmacological Reviews* 53:597-652.
13. Di Rosa M., Giroud, J.P., and Willougby, D.A. (1971). Studies of the mediators by carrageenan and turpentine. *Journal of Pathology* 104:15-29.
14. Ndebia, E.J., Kampang, R., and Nkeh-Chugang, B.N. (2007). Analgesic and anti-inflammatory properties of aqueous extract from leaves of Solanium torvum. *African Journal of Traditional, Complementary and Alternative Medicines* 4(2):240-244.
15. Tharakan, S., Nomoto, K., Mugashita, S., and Ishukawa, K. (2020). Body temperature correlates with mortality in Covid-19 patients. *Critical Care* 24(1):298.
16. Drewry, A.M., Hotchkiss, R., and Kulstad, E. (2020). Response to Body temperature correlates with mortality in covid-19. *Critical care* 24:460.
17. Huang, C., Wang, Y., Li, X., Ren, L., Zhao, J., Hu, Y., Zhang, L., Fan, G., Xu, J., Gu, X., Cheng, Z., Yu, T., Xia, J., Wei, Y., Wu, W., Xie, X., Yin, W., Li, H., Liu, M., Xiao, Y., … Cao, B. (2020). Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet* 394:497-506.
18. Manjili, R.H., Zarei, M., Habibi, M., and Manjili, M.H. (2020). Covid-19 as an acute inflammatory disease. *Journal of Immunology* 205(1):12-19.
19. Costa de Lucena, T.M., Santos, A.F., Lima, B.R., Borborema, M.E., and Silva, J.A. (2020). Mechanism of inflammatory response in associated comorbidities in COVID-19. Diabetes and metabolic syndrome. *Clinical Research and Reviews* 14:597-600.