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Original Article

Microbiology Section

Protective Action of Phosphorus 6CH in SARS-CoV-2 Spike Protein Induced Pathogenecity in *Gallus-gallus* Embryo

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ABSTRACT

Introduction: In the search of effective medicines against Coronavirus Disease-2019 (COVID-19) besides the conventional mode of treatment many medicines belonging to alternative therapeutics claimed to be effective in this disease. In homeopathy-a branch of alternative medicine, some medicines are claimed to be effective in COVID-19 after human trials.

Aim: To study whether ultradiluted preparation of Phosphorus 6CH (centesimal (C) dilutions, using Hanhemann's (H) dilution method) can protect damaging action of Delta Severe Acute Respiratory Syndrome Corona Virus 2 (SARS-CoV-2) spike protein Receptor Binding Domain (RBD) in *Gallus gallus* embryo in relation to their gross appearances, histopathological changes and cytokine changes.

Materials and Methods: The present in-vivo study was an experimental analysis carried out at the Genetic Research Laboratory of Heritage Institute of Technology, Kolkata, West Bengal, India. The whole experimental study was on 20,000 subjects in a time period of November 2021 to January 2022 and the data collected were analysed using statistical software Minitab. About 14 days old *Gallus gallus* embryonated eggs were inoculated with the antigen along with the vehicle alcohol controls. The Phosphorus 6CH was used to see whether it can prevent or cure the damaging action of the spike protein in the embryo in different experimental sets.

Results: The notable finding in this experiment is the remarkable elevated expression of Interleukin (IL)-10 gene in the curative, preventive sets as well as in the medicine control sets in comparison to antigen and alcohol control sets. In case of Transforming Growth Factor, (TGF) β1 there was enhanced expression of TGF β1 gene in the alcohol 6C set and antigen set which gets ameliorated with Phosphorus 6CH. The morbid anatomy of the embryo and the histopathological picture of the liver of the embryo also reflected similar findings in these two experimental sets. After statistical analysis it was found that there was significant correlation in between Interferon (IF) γ and IL-10 in these experimental results which appears very important.

Conclusion: The homeopathic medicine phosphorus 6CH is capable of maintaining cytokine balance in Delta SARS-CoV-2 spike protein RBD induced pathogenecity in *Gallus gallus* embryo.

Keywords: Cytokines, Histopathology, Ultradilution

INTRODUCTION

With the emergence of SARS-CoV-2 infection, the whole world had witnessed millions of death due to this dreadful virus [1]. The highly infected patients demonstrated an elevated level of proinflammatory cytokines in their blood particularly IL-6 when compared with the moderately infected patients [2]. The imbalance of cytokines results in wretched prognosis of COVID-19. Moreover, postmortem examination of the lung tissue of the COVID-19 patients revealed high infiltration of proinflammatory cells mainly macrophages along with T helper 17 (Th 17) cells [3,4]. In this context it has been also observed that with the elevated level of IL-6 in COVID-19 there is exhaustion of lymphocytes [4].

The "cytokine storm" can be considered as an activated cascade of involuntarily amplified production of cytokines due to the misbalance of immune regulation of the host system after multiple triggers [5]. The concept of "Cytokine Storm Syndrome" (CSS) have been introduced by Cron and Behrens [5]. Moreover, it has been also observed that there are elevated levels of white blood cells particularly neutrophils, C-Reactive Protein (CRP), and procalcitonin in addition to the other inflammatory indices among the Intensive Care Unit (ICU) admitted patients with COVID-19 than the non admitted counterparts [6].

Within the host system, both the innate and adaptive immune system constitutes multiple measures to combat the viral infection. It has been noticed among the SARS-CoV-2 infected patients that there is elevation in the level of chemokines and cytokines but there is decline in the level of anti-inflammatory cytokine, IL-10 [7]. In a double-blind, cluster-randomised, placebo-controlled, four parallel armed, community-based, clinical trial comprising of 20,000 subjects carried out in Kolkata within the time-span of one year has concluded that there is a possible impact of potentised Phosphorus (as per homeopathic pharmacopeia) in COVID-19. The Authors demonstrated that the Phosphorus group had the least exposure to the COVID-19 infection when compared with the groups that had received *Bryonia* or *Gelsemium* [8]. They also stated that the occurrence of unconfirmed COVID-19 cases were also least among the Phosphorus medication receiving group with statistical significance of Odds Ratio (OR) 0.1. Thus, the researchers concluded that the action of Phosphorus needs to be explored further [8].

Therefore, in this experimental investigation, authors had explored the immune-modulating efficacy of potentised phosphorus (homeopathic preparation) in fertilised chick egg model (*Gallus gallus*).

MATERIALS AND METHODS

Present study was a controlled experimental study carried out at the Genetic Research Unit of Heritage Institute of Technology within three months, November 2021-January 2022.

Chemicals: The spike protein RBD was (with mutations L452R, E484Q) of SARS-CoV-2 (B.1.617, Delta variant) was purchased from Abclonal, USA. This recombinant protein was synthesised within HEK293 (Human Embryonic Kidney 293 cell line) expression

system. This particular target protein with sequence (Arg319-Phe541) of RBD which was tagged with poly-histidine at the C-terminus was produced. Phosphorus 6CH was purchased from Government approved Alternative medicine producing company-"HAPCO, India". The 14th day fertilised chick eggs was purchased from the State poultry farm, Kolkata on which the experimental process had been carried out.

Study Procedure

Different experimental sets were prepared where each set consist of three eggs [9]. The sets were: Control set comprises of 14th day embryonated eggs; Solvent (Alcohol) control where the eggs are challenged with 70% v/v of alcohol of molecular biology grade; Alcohol 6 CH (potentized homeopathic preparation) control where the eggs were challenged with the potentised homeopathic preparation; Medicine control (the fertilised eggs were challenged with Phosphorus of homeopathic potency 6CH); Antigen control (the eggs were challenged with spike protein (RBD) of SARS-CoV-2 of concentration 10 µg/mL dissolved in phosphate buffer saline. To evaluate both the preventive and curative action of the medicine against the pathological changes by the spike protein two combinations were investigated.

Preventive set: Ultradiluted phosphorus (potency 6C, homeopathic preparation) was initially inoculated and thereafter spike protein antigen within a time gap of one hour in between.

Curative set: Spike protein antigen was initially inoculated and thereafter ultradiluted phosphorus (potency 6C, homeopathic preparation) within a time gap of one hour in between.

The results of different experimental sets are given below. The abbreviated names of the different sets are also given below.

- ES 1: Medicine Control (Phosphorus 6CH)
- ES 2: Phosphorus 6CH challenged by Antigen
- ES 3: Antigen challenged by Phosphorus 6CH
- ES 4: Spike protein antigen challenged embryo
- ES 5: Vehicle control (Alcohol)
- ES 6: Vehicle control (Alcohol 6CH)

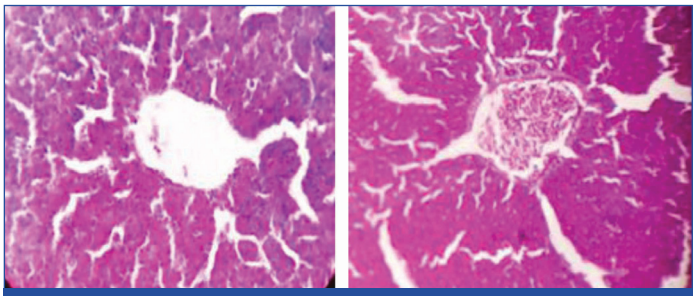
Inoculation of eggs: The spike protein antigen and the medicine were inoculated via the amniotic route in the 14th day embryonated eggs. Prior to inoculation, the eggs were cleansed with water and the air sac was marked with a marker. The air sacs were then disinfected with 70% ethyl alcohol followed by povidone iodine solution twice and then the sites were punctured with sterile needle. The volume of the inoculum within the eggs was 100 µL and the inoculation was done with sterile 1 mL syringes [9].

Collection of allantoic fluid: The embryonated eggs were harvested on the 17th day with sterile scissors and forceps and the allantoic fluid was collected with sterile 5 mL syringes into a sterile sample collecting containers. In a pilot study it was observed that collection of allantoic fluid is very easy and the changes in allantoic fluid are equivalent to changes in the amniotic fluid [9]. The fluids were stored at-80°C for further study. Liver tissue samples were collected and preserved within 10% formol saline for histopathological study. The embryos were also observed for any gross pathological changes [9].

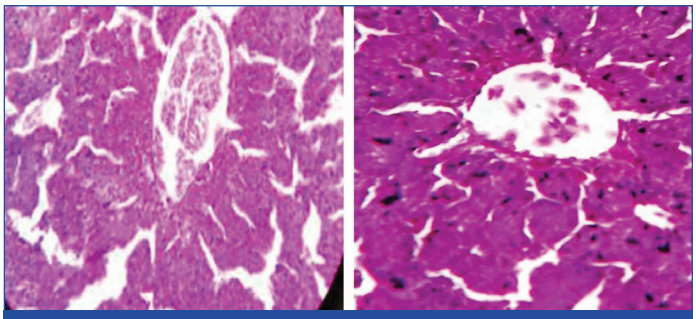
Molecular biological study: The total Ribonucleic Acid (RNA) was extracted using RNA isoplus and the whole extraction was carried following the protocol of the manufacturer (Takara, USA). The total RNA was quantified using Ultraviolet- vis spectrophotometer (Agilent) using the absorbance ratio at 260 nm by 280 nm. The total RNA was then converted to cDNA using cDNA reverse transcriptase synthesis kit (Bio-Rad, USA). The cDNA were utilised to perform the semiquantitative gene expression analysis of the following cytokine parameters namely, IFN α,β,γ; IL-6, IL-8, IL-10, IL 1β; TGF β1 and β3 using the Real time Polymerase Chain Reaction (PCR) (Bio-Rad, CFX-96 instrument, USA) against the house keeping gene β-actin [10,11]. The semiquantitative gene expression values were quantified using the formula 2^(-ΔΔCt) where $\Delta\Delta Ct=\Delta Ct_1-\Delta Ct_2$, here the following symbols denotes

ΔΔCt=ΔCt (treated sample)-ΔCt (untreated sample); ΔCt=Ct (gene of interest)-Ct (housekeeping gene); ΔCt=Ct (gene of interest)-Ct (housekeeping gene). Ct stands for cycle threshold.

Histopathological study: The tissue samples from both sets were grossed and processed for the histopathological analysis. The tissue samples were stained using the haematoxylin and eosin staining (H&E staining) following the standard protocol [Table/Fig-1-4] [12].



[Table/Fig-1]: Histopathological section shows features of normal liver of the embryonated eggs. **[Table/Fig-2]:** Histopathological section shows features of embryonated egg liver challenged with the antigen showing micro clots and necrosis (H&E, 400X). (Images from left to right)



[Table/Fig-3]: Histopathological section shows features of embryonated egg liver in pre-experimental set showing micro clots and mild necrotic changes (magnification at 400X). **[Table/Fig-4]:** Histopathological section shows normal features of embryonated egg liver in post-experimental set (H&E, 400X). (Images from left to right)

STATISTICAL ANALYSIS

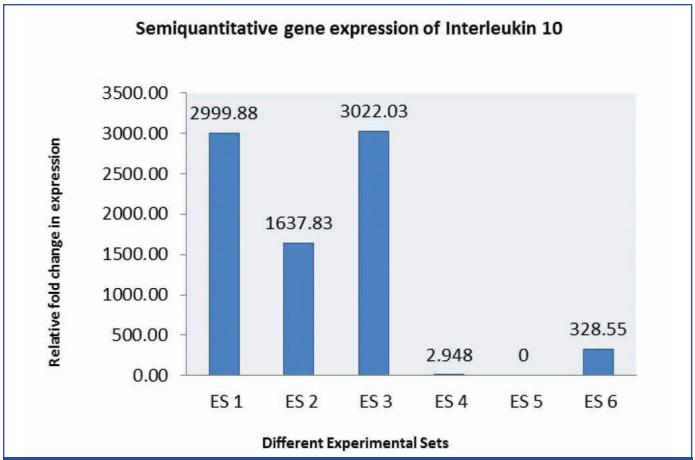
Python statistical software was used to analyse the data and was used to calculate the Pair-wise Pearson Correlations values, analysis of variance and regression equations.

RESULTS

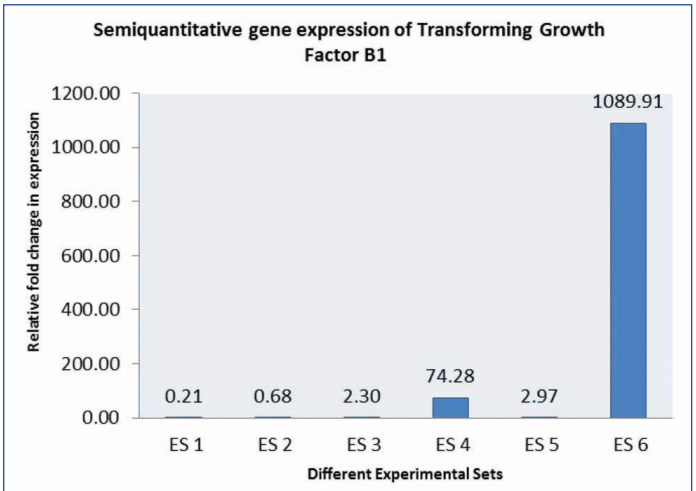
The semiquantitative expressions of the cytokines have been represented in the bar chart diagrams. The most important finding in this experiment was the remarkable elevated expression of IL-10 gene in the curative, preventive sets as well as in the medicine control sets in comparison to antigen and alcohol control sets [Table/Fig-5]. In case of TGF β1 there was enhanced expression of TGF β1 gene in the alcohol 6C set and antigen set which gets ameliorated with Phosphorus 6CH [Table/Fig-6].

It was also observed that IFN α gene expression was enhanced significantly with alcohol in different experimental sets [Table/Fig-7]. Similarly IFN γ gene expression was found markedly increased by alcohol 6C [Table/Fig-8]. It has also been noticed that there is alcohol induced IL-6 gene expression in the medicine control, preventive and curative sets [Table/Fig-9].

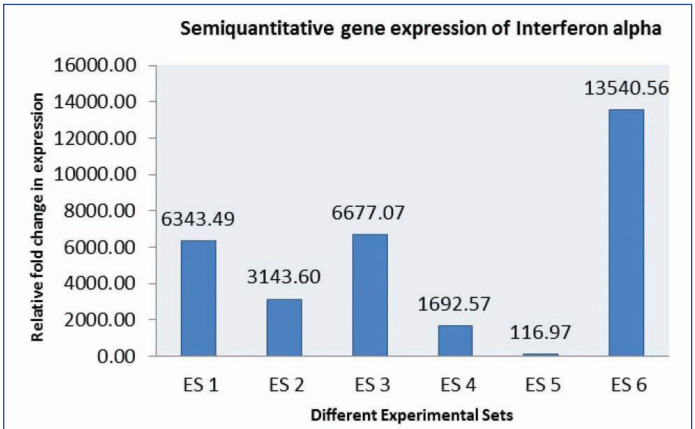
The [Table/Fig-10 a-f] represents the gross macroscopic appearance of the embryo after harvesting. The embryos which received the spike protein antigen were dead and putrefied. The preventive and curative sets and the medicine control set showed healthy growth of the embryos but evidences of haemolysis was observed in all the



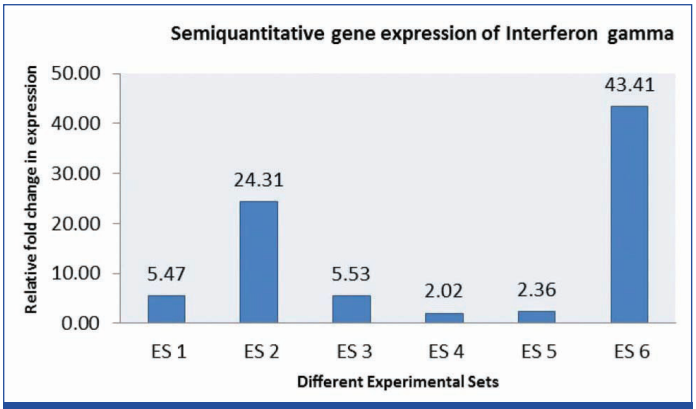
[Table/Fig-5]: IL-10 gene expressions in terms of normal fold increase among different experimental sets.



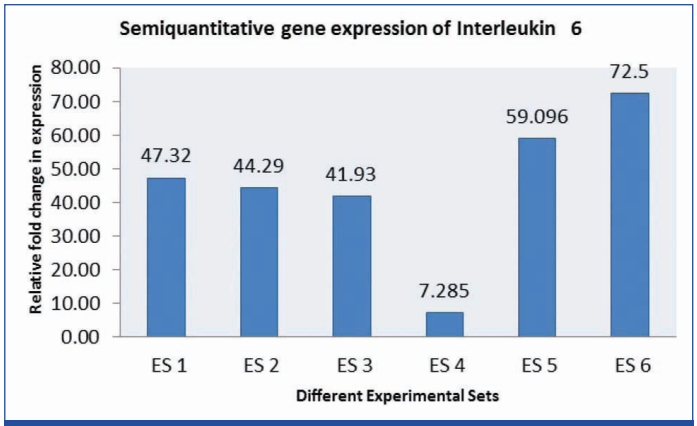
[Table/Fig-6]: TGF β1 gene expressions in terms of normal fold increase among different experimental sets.



[Table/Fig-7]: IFN α gene expressions in different experimental sets.



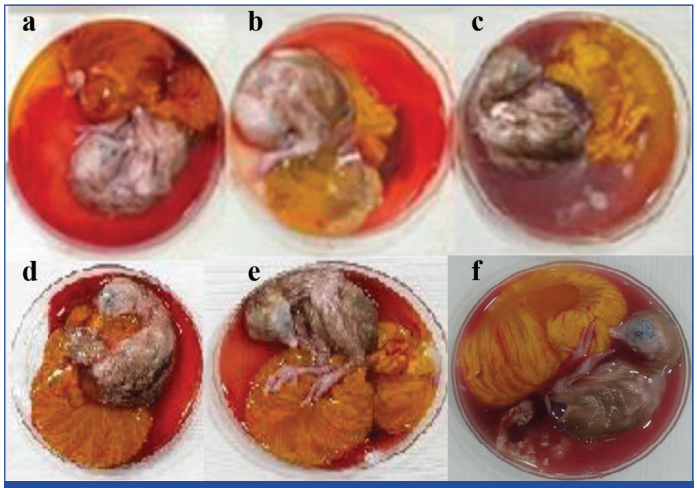
[Table/Fig-8]: IFN γ gene expressions in terms of normal fold increase among different experimental sets.



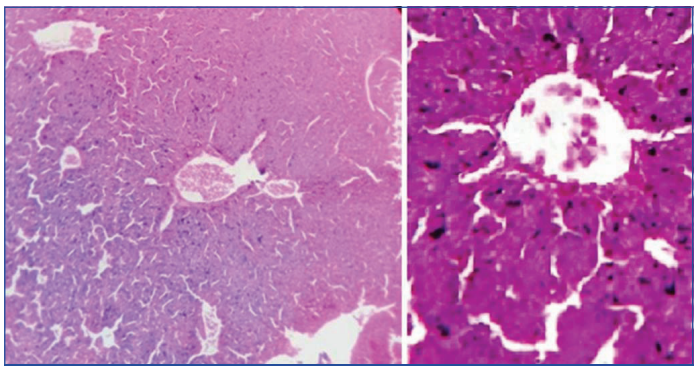
[Table/Fig-9]: IL-6 gene expressions in terms of normal fold increase among different experimental sets.

sets. Haemolysis was comparatively less in the curative set with respect to the other sets.

In histopathological study in the curative set the hepatocytes were normal and there was no infiltration of inflammatory cells or clots [Table/Fig-10 g]. With antigen there were gross necrosis with clots in the central veins. In preventive set some necrotic changes and clots in the central vein persisted. With alcohol 6CH some hepatocytes showed necrotic changes.



[Table/Fig-10]: Gross appearance (representative) of embryo in different experimental sets. (a) Control; healthy well developed embryo; (b) Alcohol 6CH; healthy embryo; (c) Spike protein antigen challenged embryo; The embryo was dead and putrfied; (d) Preventive set (Phosphorus 6CH challenged by Antigen); (e) Curative set (Antigen challenged by Phosphorus 6CH); (f) Medicine Control (Phosphorus 6CH). The preventive and curative sets and the medicine control set showed healthy growth of the embryos but evidences of haemolysis was observed in all the sets.



[Table/Fig-10 g]: Histopathological section of liver in curative set showing normal hepatocytes without any clots in the central vein (H&E, left 100X, right 400X).

There was significant correlation between IFN γ and IL-10 in these experimental results. Other correlation values and their significance levels are given in [Table/Fig-11,12]. The statistical analysis showed that there was positive Pearson Correlation in between different pairs of cytokines with p-values <0.05.

Groups	I	II	III	IV	V	VI	VII
II	0.039						
III	0.373	0.257					
IV	0.384	0.867	0.198				
V	0.481	-0.241	0.777	-0.249			
VI	0.051	0.998	0.284	0.859	-0.216		
VII	0.008	0.999	0.222	0.855	-0.277	0.996	
VIII	0.869	-0.025	0.683	0.238	0.715	0.009	-0.066

[Table/Fig-11]: Correlations between group.
Here, I-Interferon alpha (IFN α), II-Interferon beta (IFN β), III-Interferon gamma (IFN γ), IV-Interleukin 8 (IL-8), V-Interleukin 10 (IL-10), VI-Interleukin 1 beta (IL-1β), VII-Transforming growth factor beta 1 (TGF-β1), VIII-Interleukin 6 (IL-6)

Sample 1	Sample 2	Correlation	95% CI for ρ	p-value
II	I	0.039	(-0.642, 0.685)	0.921
III	I	0.373	(-0.387, 0.831)	0.322
IV	I	0.384	(-0.376, 0.835)	0.307
V	I	0.481	(-0.269, 0.868)	0.190
VI	I	0.051	(-0.635, 0.692)	0.896
VII	I	0.008	(-0.660, 0.668)	0.985
VIII	I	0.869	(0.486, 0.972)	0.002
III	II	0.257	(-0.491, 0.787)	0.505
IV	II	0.867	(0.479, 0.972)	0.002
V	II	-0.241	(-0.780, 0.504)	0.532
VI	II	0.998	(0.988, 1.000)	0.0001
VII	II	0.999	(0.993, 1.000)	0.0001
VIII	II	-0.025	(-0.678, 0.650)	0.950
IV	III	0.198	(-0.537, 0.762)	0.609
V	III	0.777	(0.234, 0.951)	0.014
VI	III	0.284	(-0.468, 0.798)	0.458
VII	III	0.222	(-0.519, 0.772)	0.566
VIII	III	0.683	(0.035, 0.927)	0.042
V	IV	-0.249	(-0.783, 0.498)	0.519
VI	IV	0.859	(0.455, 0.970)	0.003
VII	IV	0.855	(0.442, 0.969)	0.003
VIII	IV	0.238	(-0.506, 0.779)	0.537
VI	V	-0.216	(-0.770, 0.523)	0.577
VII	V	-0.277	(-0.795, 0.475)	0.471
VIII	V	0.715	(0.097, 0.935)	0.030
VII	VI	0.996	(0.979, 0.999)	0.001
VIII	VI	0.009	(-0.659, 0.669)	0.982
VIII	VII	-0.066	(-0.700, 0.625)	0.866

[Table/Fig-12]: Pairwise Pearson Correlations.
Here, I-Interferon alpha (IFN α), II-Interferon beta (IFN β), III-Interferon gamma (IFN γ), IV-Interleukin 8 (IL-8), V-Interleukin 10 (IL-10), VI-Interleukin 1 beta (IL-1β), VII-Transforming growth factor beta 1 (TGF-β1), VIII-Interleukin 6 (IL-6)

DISCUSSION

As it is known to all of us, that IFNs are the cytokines which acts as the frontline defense mechanism of our body against viruses. These chemicals are secreted by cells which are infected by virus [13]. The IFNs secreted by cells in turn stimulates or up-regulates many other IFNs genes that overall causes blocking of the replication of the virus at several steps and thereby depicts antiviral action [13]. When our cell achieves the antiviral state, there occurs cross-talk between the pathways that regulates the apoptotic mechanism and the signaling pathway of IFNs. In turn there are other associated effects observed such as up-regulation of the inflammatory mechanism along with the stress response program at the cellular level [13]. In present study, there was increased up-regulation of both IFNs α and γ in the preventive and curative treatment sets respectively [Table/Fig-1,2]. The markedly increased IFN gene expressions with alcohol 6CH were modified by the medicine to a moderate level of increased IFN gene expression probably for homeostasis. The role of IL-6 is considered to be extremely important as it has already been studied that among the COVID-19 hypoxic patients, the concentration of IL-6 is quite high and if the level rises above 24 pg/Ml the condition of the patient is critical [2]. Therefore, IL-6 is considered to be an important marker for serious COVID-19 infected patients and also used to monitor patient's prognosis. In this respect, another study on infection of bronchitis virus (IBV) in HD11 chicken macrophage cell line and chicken peripheral blood mononuclear cell-derived macrophages (PBMCs-Mϕ) with a multiplicity of infection of 10 revealed that among varied high level of gene expressions such as TLR3, IFN-α, CCL4, MIF, IL-1β, IL-6 also demonstrated high gene expression value in the virus infected cells [14]. However, present study have observed an controlled IL-6 gene expression in the medicine control, preventive and curative sets in comparison to the alcohol 6CH. The anti-inflammatory cytokine IL-10 increases among the patients suffering from COVID-19 condition. This particular cytokines plays the role of lowering down the hyper inflammation process and prevents the damage of tissues [15]. The most notable result obtained in present study was that there was a remarkable elevated expression of IL-10 in the medicine control, curative and preventive sets in comparison to antigen and alcohol control sets. Another important observation was noticed with TGF β which was considered to be a high-flying regulator of immune reactions [16]. It is also responsible for fibrosis, which can be observed among severe COVID-19 patients [16]. However, in present study there was an enhanced expression of TGF β1 gene in the alcohol 6C set and antigen set which gets ameliorated with Phosphorus 6CH (medicine control).

In the statistical analysis, it was found that there was significant correlation in between IFN γ and IL-10 in these experimental results which appears very important. These two cytokine changes may protect the person from cytokine imbalance. In this study in both preventive and curative sets although the main proinflammatory cytokine gene expression is increased about 40 folds but the main anti-inflammatory cytokine gene expression is much more increased (in preventive set 1637 fold; in curative set 3022 fold) to counteract the possible detrimental effects caused by IL-6 gene expression. In this way, it can produce a physiological balance without any harmful effect.

Limitation(s)

Since, the eggs were obtained from vaccinated chickens due to which it may carry antibodies that might interfere in the gene expression assay and some of the bacteria like *Mycoplasma* and *Salmonella* can pass from chicken to their eggs [17].

CONCLUSION(S)

The homeopathic medicine Phosphorus 6C was found capable of balancing cytokine alterations induced by Delta SARS-CoV-2 spike protein RBD in *Gallus gallus* embryonated eggs.

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REFERENCES

[1] Machhi J, Herskovitz J, Senan AM, Dutta D, Nath B, Oleynikov MD, et al. The natural history, pathobiology, and clinical manifestations of SARS-CoV-2 infections. J Neuroimmune Pharmacol. 2020;15(3):359-86.

[2] Sabaka P, Koščálová A, Straka I, Hodosy J, Lipták R, Kmotorková B, et al. Role of interleukin 6 as a predictive factor for a severe course of COVID-19: Retrospective data analysis of patients from a long-term care facility during COVID-19 outbreak. BMC Infect Dis. 2021;21(1):01-08.
[3] Tabary M, Khanmohammadi S, Araghi F, Dadkhahfar S, Tavangar SM. Pathologic features of COVID-19: A concise review. Pathol Res Pract. 2020;216(9):153097.
[4] dos Santos Miggiolaro AFR, da Silva Motta Junior J, de Paula CBV, Nagashima S, Malaquias MAS, Carstens LB, et al. COVID-19 cytokine storm in pulmonary tissue: Anatomopathological and immunohistochemical findings. Respir Med Case Rep. 2020;31:101292.
[5] Cron RQ, Behrens EM, editors. Cytokine storm syndrome. Springer Nature; 2019; Edition 1: XXI, 617.
[6] Li W, Lin F, Dai M, Chen L, Han D, Cui Y, et al. Early predictors for mechanical ventilation in COVID-19 patients. Ther Adv Respir. 2020;1753466620963017.
[7] Lu L, Zhang H, Dauphars DJ, He YW. A potential role of interleukin 10 in COVID-19 pathogenesis. Trends Immunol. 2021;42(1):03-05.
[8] Mukherjee SK, Ganguly S, Das S, Chatterjee KK, Naskar KK, Dey S, et al. Homeopathic medicines used as prophylaxis in Kolkata during the COVID-19 pandemic: A community-based, cluster-randomized trial. Homeopathy. 2022;111(2):97-104. Doi: 10.1055/s-0041-1734026. PMID: 34715718.
[9] Guy JS. Isolation and propagation of coronaviruses in embryonated eggs. Methods Mol Biol. 2008;454:109-17.
[10] Chakraborty U, Sinha M, Bhattacharjee A, Nayak D, Khurana A, Manchanda RK, et al. Suppression of viral load by belladonna 200c through modulation of TLR and type-IIFN signalling pathways. Int J Biol Med Res. 2019;10(01):6635-40.
[11] Marone M, Mozzetti S, De Ritis D, Pierelli L, Scambia G. Semiquantitative RT-PCR analysis to assess the expression levels of multiple transcripts from the same sample. Biol Proced Online. 2001;3(1):19-25.
[12] Chakraborty U, Katoch S, Sinha M, Nayak D, Khurana A, Manchanda RK, et al. Changes in viral load in different organs of Japanese Encephalitis virus-infected chick embryo under the influence of Belladonna 200C. Indian J Res Homoeopathy. 2018;12:75-80.
[13] Katze MG, He Y, Gale M. Viruses and interferon: A fight for supremacy. Nat Rev Immunol. 2002;2(9):675-87.
[14] Sun X, Wang Z, Shao C, Yu J, Liu H, Chen H, et al. Analysis of chicken macrophage functions and gene expressions following infectious bronchitis virus M41 infection. Veterinary Research. 2021;52(1):01-05.
[15] Han H, Ma Q, Li C, Liu R, Zhao L, Wang W, et al. Profiling serum cytokines in COVID-19 patients reveals IL-6 and IL-10 are disease severity predictors. Emerg Microbes & Infect. 2020;9(1):1123-30.
[16] Ferreira-Gomes M, Kruglov A, Durek P, Heinrich F, Tizian C, Heinz GA, et al. SARS-CoV-2 in severe COVID-19 induces a TGF-β-dominated chronic immune response that does not target itself. Nat Commun. 2021;12(1):01-04.
[17] Saif YM. Diseases of Poultry. John Wiley & Sons; 2009;13.

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