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COMPARATIVE ACTION OF ALTERNATIVE MEDICINES *ARSENICUM ALBUM* 30CH AND *PHOSPHORUS* 30CH FOR BALANCING CYTOKINES GENE EXPRESSIONS IN SARS-COV-2 SPIKE PROTEIN INDUCED PATHOLOGICAL CHANGES

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Alternative medicines may play a significant role both in terms of protective and curative actions against viral diseases like COVID-19. Some clinical trials with alternative medicines against COVID-19 showed controversial outcomes. Therefore, in this study, we explored the efficacy of two such alternative medicines namely Arsenic album 30C and Phosphorus 30C on fertilized chick (Gallus gallus domesticus) egg model against pathological changes induced by RBD spike protein (S1) antigen of SARS- CoV- 2.

In this study, 14th-day-old fertilized eggs were challenged with the S1 protein along with the medicines and different controls. On harvesting after 48 h, the morbid anatomy was observed followed by collection of the allantoic fluid for molecular biology assay. Cytokine gene expressions namely Interleukins – IL -6, IL-8, IL-10, Interferons – IFN α, β, γ; and Transforming growth factor – TGF β1 were studied by RT-PCR along with the housekeeping gene β actin. The results of this study indicated a significant role of these medicines against RBD S1 antigen-induced pathogenic changes in egg models.

Keywords: *Gallus gallus domesticus, Interleukins, Interferons, Transforming growth factor, Arsenic album 30C, Phosphorus 30C*

INTRODUCTION

The severe acute respiratory syndrome (SARS) coronavirus-2 belonging to the family of Coronaviridae is a new strain responsible for the havoc respiratory infections that took birth in Wuhan, China and thereafter the entire globe suffered from millions of death due to the infection caused by the virus[1]. The clinical symptoms of the disease can range from mild to severe form of illness, among them the need of hospitalization is required by the moderate or severe cases, requiring invasive or non-invasive ventilation. The patients were also administered with antivirals, steroids, antipyretics and antibiotics. It has been also observed that few patients suffering from complicated conditions required plasma exchange therapy and application of immune-modulatory drugs[2].

At the time of second wave of COVID-19, Ministry of Ayush, Government of India has advised the administration of *Arsenicum Album* of potency 30CH which would act as immune booster and also as prophylactic medicine against the disease and it would reduce the increasing cases of hospitalization (Ministry of Ayush guidelines)[3]. In our previous research study we have shown the action mechanism of *Arsenic album* 6C in fertilized *Gallus gallus* egg model following induction of pathophysological changes with spike protein of Delta SARS-CoV-2 variant. The study demonstrated prominent change, IL-10 cytokine gene expression was highly increased in the control medicine set along with the pre and post treatment sets. Histological findings also depicted that *Arsenic album* 6C have hepato-protective action especially in the pre- and post experimental sets[4]. A prospective parallel cluster cohort study was carried out in the COVID -19 containment areas of Delhi, where cases of age 5 years or above were given with five medicated pills of *Arsenicum*

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album 30C and the other group below 5 years of age were given the dosage of two medicated pills. The researchers concluded that *Arsenicum album* 30C was able to provide certain level of protection against the laboratory confirmed COVID-19 cases in that containment zone, however randomized controlled trials are necessary to confirm the above findings[5].

Similarly, prior literature survey have shown that in a double-blind, cluster-randomized, placebo-controlled, four parallel arms, community-based, clinical trial, carried out by Mukherjee, *et al*., 2022 on a population of 20,000 living at Kolkata was administered with three medicines namely *Bryonia alba* 30C, *Gelsemium sempervirens* 30C, and *Phosphorus* 30C or indistinguishable-looking placebo, for 3 or 6 days for children or adult respectively[6]. The researchers in the findings reported that the group which received medicine *Phosphorus* 30C had suffered the minimum from COVID-19 or the cases are mostly un-confirmed when compared with other two groups receiving *Bryonia alba* 30C and *Gelsemium sempervirens* 30C [6]. However, in a clinical trial study in Brazil they found no benefit of the alternative medicine over the placebo[7].

Thus, in the present research study we have explored the action mechanism of two such homeopathic medicines namely *Arsenicum album* and *Phosphorus* of potency 30C on embryonated chick egg model, *Gallus gallus domesticus*.

MATERIALS AND METHODS

The receptor binding domain (RBD, L452R, E484Q) of the spike protein of antigen SARS-CoV -2 (B.1.617, Delta variant) was purchased from Abclonal, USA (Product code: WH192258, Lot: 9621050601, Cat. No. RPO2266). The medicines *Arsenic album* 30C and *Phosphorus* 30C were purchased from an Indian government authorized alternative medicine company, HAPCO, India.

14th day fertilized hen cell eggs were purchased and cleaned with distilled water. After candling procedure, the air sacs were marked with marker and that region was cleaned with 70% alcohol followed by povidone iodine (10% w/v) and again by 70% alcohol. 100 µL of inoculums were inoculated using sterile 1mL syringe via the amniotic route. The following sets were prepared – Control eggs (3 such); eggs inoculated with 70% alcohol (3 such); eggs inoculated with viral antigen (3 such); eggs inoculated with medicine control (3 such each for *Arsenic album* 30C and *Phosphorus* 30C respectively); Post-treatment sets - eggs inoculated with antigen challenged by medicines after 1 hour gap; and Pre-treatment sets - eggs inoculated with medicines challenged by antigen after 1 hour gap[8].

The eggs were incubated at 38°C at 60 – 80% humidity for another 48 hours and then harvested at 17th day with sterile scissors and forceps to collect the allantoic fluid [8] (Ref. **Fig. 1)**.

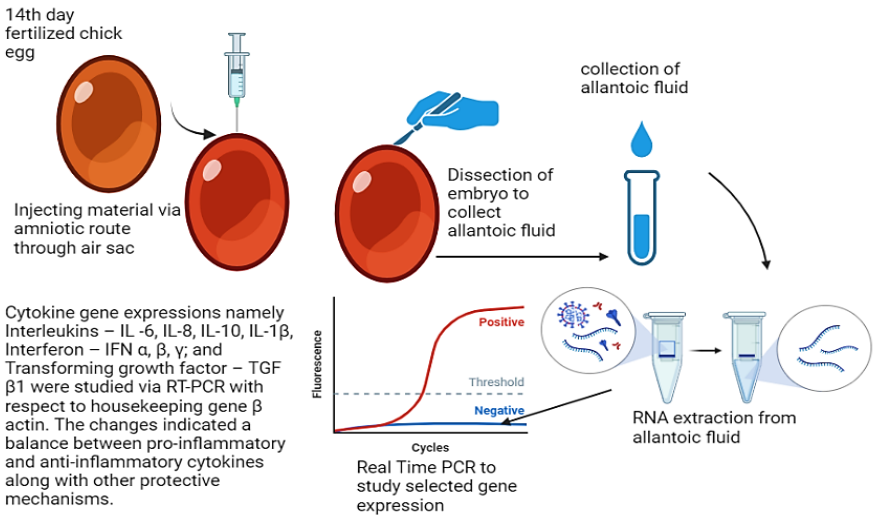

Fig. 1: Graphical representation of the methodology.

Molecular biology Study

The total RNA was isolated from allantoic fluid using RNA isoplus or Trizol following manufacturer's protocol. Purity of RNA was measured using spectrophotometer by A260/ 280 ratio. cDNA was synthesized using iscript reverse transcriptase kit (Bio-Rad, USA). Relative fold gene expression study was done using RT PCR, (CFX 96 model, Bio-Rad, USA) for the following genes – Interferons α, β, γ; Interlekins – IL-6, IL-8, IL-10, IL-1β, Transforming growth factor, TGF β1, against housekeeping gene β- actin. The 2^-ΔΔct value were calculated for evaluating the relative fold gene expression[8].

Morbid anatomy study

The embryos were closely observed to analyze their different aspects of morbid anatomy changes.

Statistical analysis

The data was analyzed by two way ANOVA using the statistical tool GraphPad Prism version 9.5.3. Two way ANOVA was done to find out the variance between column factors and row factors of all the cytokine parameters.

RESULTS AND DISCUSSION

Results

Morbid anatomy study

The morbid anatomy showed that with direct antigen, the embryo was dead and slightly putrified while with alcohol exposure the embryo were slightly swollen but both in the medicine control sets, preventive and curative sets the embryos appears to be normal like the control set (refer **Fig. 5A and 5B**).

Comparative Cytokine Study

IFNs play a significant role in anti-viral response as the first line of defense by our immune system, and in turn, they inhibit the replication of viruses through several pathways. In the case of IFN α, the antigen itself could not induce the particular cytokine gene expression, whereas *Phos* 30C could remarkably induce the gene expression of IFN α in both the preventive, curative sets and also in the *Phos* 30C control set. When we observed the medicine control sets of *Ars* 30C, its preventive and curative sets, the gene expression got moderately increased in all the sets. Thus, it can be understood that *Phos* 30C could induce a higher anti-viral response in comparison to *Ars* 30C, so this particular finding indicated the promising curative aspect of *Phos* 30C than *Ars* 30C **(Fig. 2A)**. There was no stimulation of IFN α by vehicle control (ethanol). IFN β got induced with the inoculation of antigen, and it was observed that with medicine *Phos* 30C alone, the gene expression level is much higher when compared with *Ars* 30C. In the curative sets of both *Phos* 30C and *Ars* 30C the IFN β gene expressions were found to be comparative. The alcohol control itself could induce the gene expression of IFN β up to a moderate level which was comparative with the curative action of both medicines. However, in the preventive set, it was observed that *Phos* 30C induced comparatively much higher cytokine gene expression than *Ars* 30C. This finding also highlights the higher efficacy of *Phos* 30C than *Ars* 30C **(Fig. 2B)**. In the case of IFN γ there was no such noticeable change in the preventive and curative sets of medicine *Ars* 30C and *Phos* 30C. Although medicine *Phos* 30C alone could stimulate the gene expression of IFN γ but with the addition of antigen it got suppressed **(Fig. 2C).**

IL-8 is a chemo-attractant cytokine produced by activated neutrophils in the inflammatory region. In the preventive set of *Phos* 30C the gene expression of IL-8 had remarkably increased whereas no such noticeable change had been observed with the *Ars* 30C medicine. With the application of *Ars* 30C, the changes of IL-8 gene expressions were in control in all the experimental sets, therefore, *Ars* 30 showed better efficacy in controlling the severity of the disease **(Fig. 3A)**. Vehicle control showed marginal influence upon the gene expression of IL-8.

With the application of *Phos* 30C medicine, the gene expression of IL-10 was increased considerably in the medicine control set, in the curative set the changes in IL-10 gene expression were similar for both the medicines. In the preventive set, *Ars* 30C could increase gene expression significantly in comparison to *Phos* 30C. As IL-10 is considered to be a potent regulator of

inflammatory response and prevents damage of the tissues, therefore, here *Ars* 30C holds promising preventive action. In the curative sets of both the medicines, the changes were similar **(Fig. 3B)**. Vehicle control showed marginal influence on the gene expression of IL-10.

In the medicine control set *Phos* 30C had enhanced the gene expression of IL-6 when compared to *Ars* 30C. With *Ars* 30C, in the curative set, the expression of the IL-6 gene had decreased considerably. Another important finding was that there was considerably higher gene expression of IL-6 in all the experimental sets with *Phos* 30C which were in a controlled manner with medicine *Ars* 30C. This finding also highlighted the better efficacy of *Ars* 30C in terms of controlling the cytokine surges that cause detrimental effects on the health of the patients **(Fig. 3C)**.

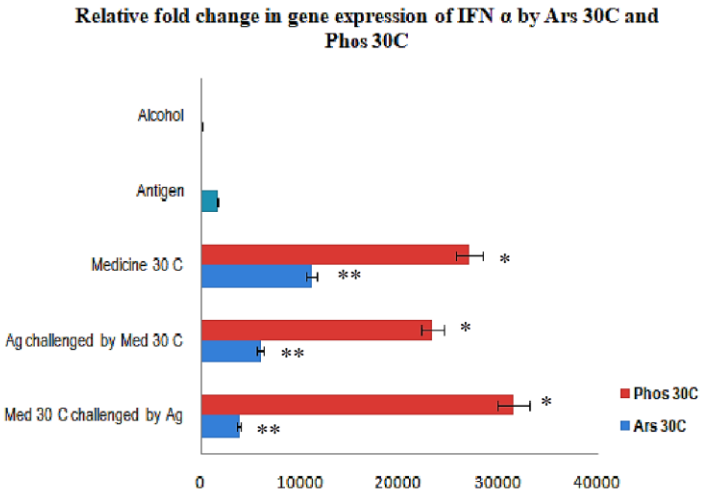

Fig. 2A: Graph showing the relative fold IFN α gene expression changes in different experimental sets. The markedly increased gene expressions were found in all experimental sets with *Phos* 30C. With *Ars* 30C the gene expressions were moderately increased. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

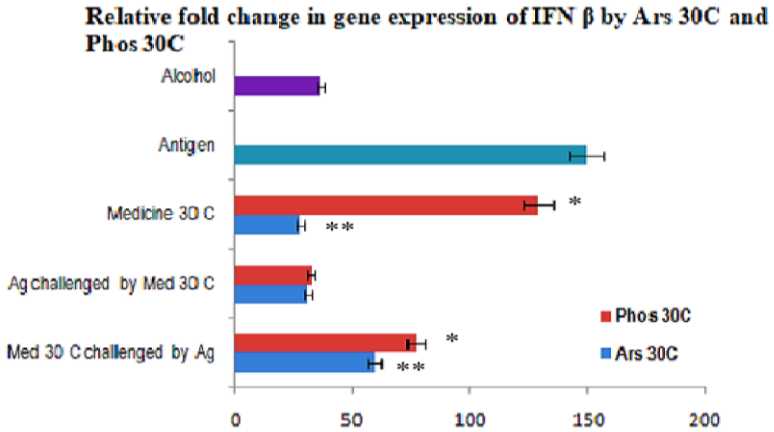

Fig. 2B: Graph showing the relative fold IFN β gene expression changes in different experimental sets. The markedly increased gene expressions by the antigen were mitigated by both the medicines in a significant way although alone the medicine *Phos* 30C also increased the gene expression significantly. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

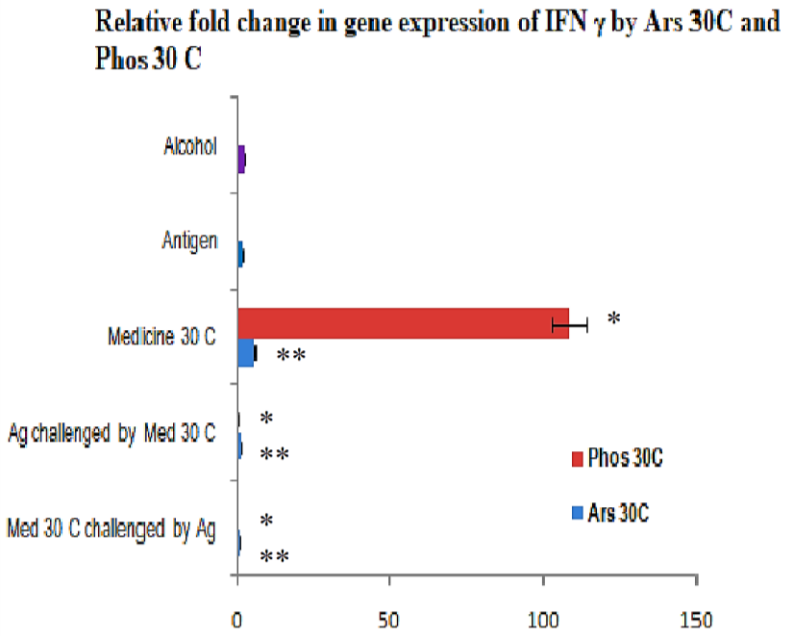

Fig. 2C: Graph showing the relative fold IFN γ gene expression changes in different experimental sets. Only the medicine *Phos* 30C markedly increased gene expressions. In all other sets and with *Ars* 30C alone the gene expression was almost normal. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

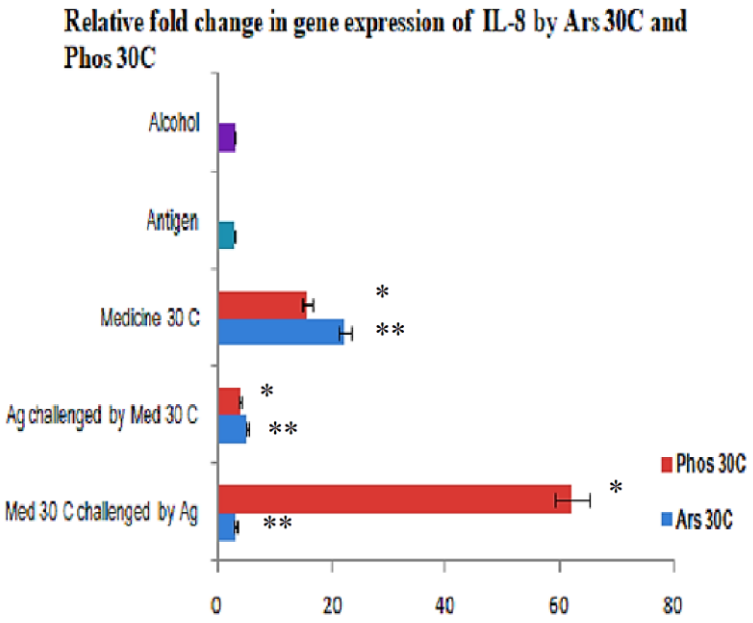

Fig. 3A: Graph showing the relative fold IL-8 gene expression changes in different experimental sets. In the preventive set of *Phos* 30C the gene expression of IL-8 had remarkably increased whereas no such noticeable change had been observed with the *Ars* 30C medicine. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

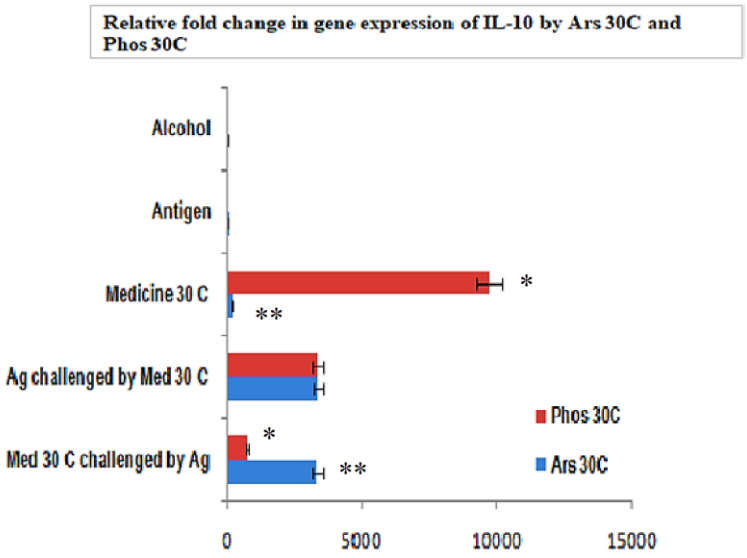

Fig. 3B: Graph showing the relative fold IL-10 gene expression changes in different experimental sets. With application of *Phos* 30C medicine the gene expression of IL-10 was increased considerably in the medicine control set, in the curative set the changes in IL-10 gene expression was similar but in the preventive set *Ars* 30C could increase the expression significantly in comparison to *Phos* 30C. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

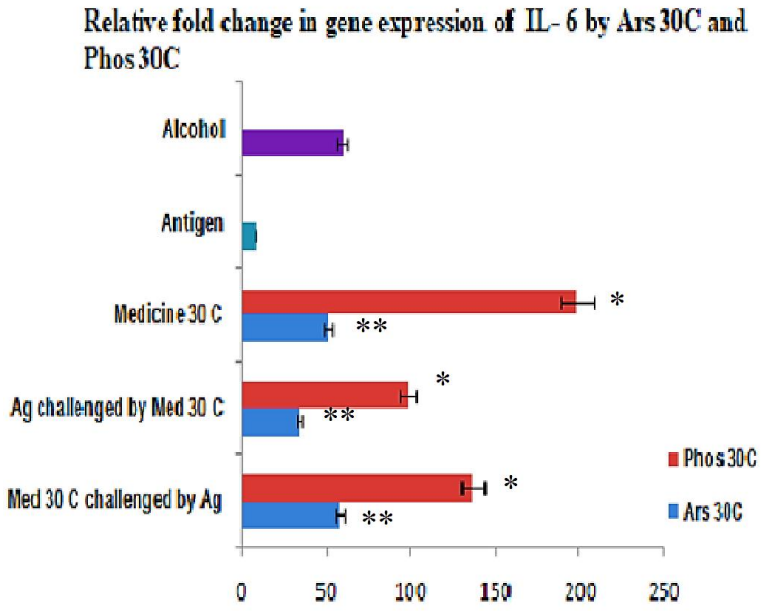

Fig. 3C: Graph showing the relative fold IL-6 gene expression changes in different experimental sets. In the medicine control set *Phos* 30C had enhanced the gene expression when compared to *Ars* 30C. With *Ars* 30C, in the curative set, the expression of the gene had decreased considerably. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

With the inoculation of antigen the gene expression of TGF β was increased, however, both in the curative and preventive set of *Ars* 30C and *Phos* 30C, the increased TGF β gene had been down-regulated. Thus ameliorative action is observed for both medicines **(Fig. 4)**.

Statistical Analysis

Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. The data were found to be statistically significant with P value < 0.001 with alpha level considered to be 0.05 (Supplementary File, Table 1 – 3). Therefore, the variation in between column factors and row factors were statistically significant.

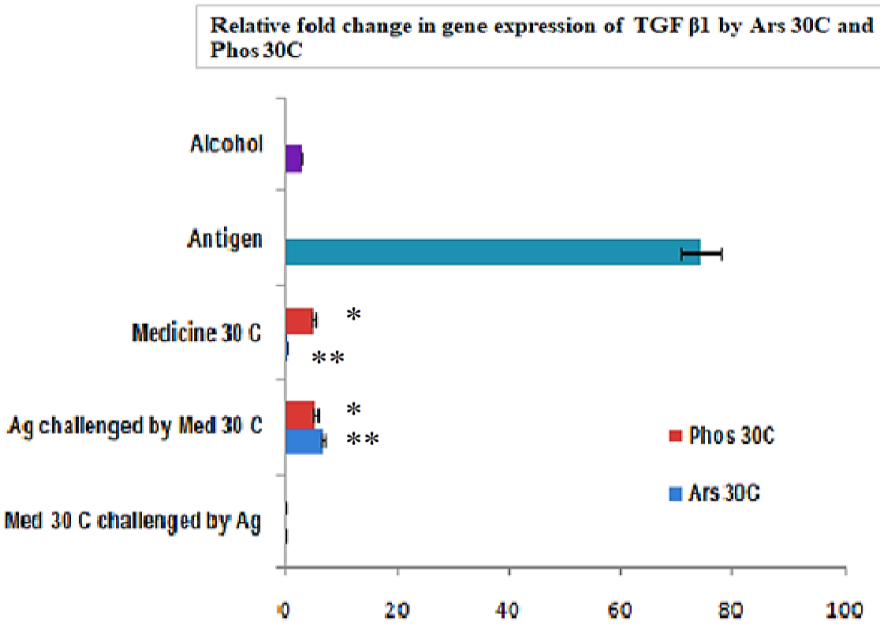

Fig. 4: Increase in gene expression of TGF- β1 by direct Ag was significantly decreased by *Ars* 30C. Although direct Ag increases the gene expression of TGF- β1 but after addition of *Phos* 30C it was markedly decreased. The changes with both medicines were comparable. Two way ANOVA Analysis (interaction between row factors and column factors) of Mean of Phos 30C and Ars 30C, Mean of Ag challenged by Phos 30C and Ars 30C and Mean of Ars 30 C challenged by Ag vs Mean of Phos 30 C challenged by Ag were found to be significant at P value < 0.0001, Alpha value was 0.05. (* denotes the changes in the data of Phos 30C experimental sets; ** denotes the changes in the data of Ars 30C experimental sets).

Morbid anatomy

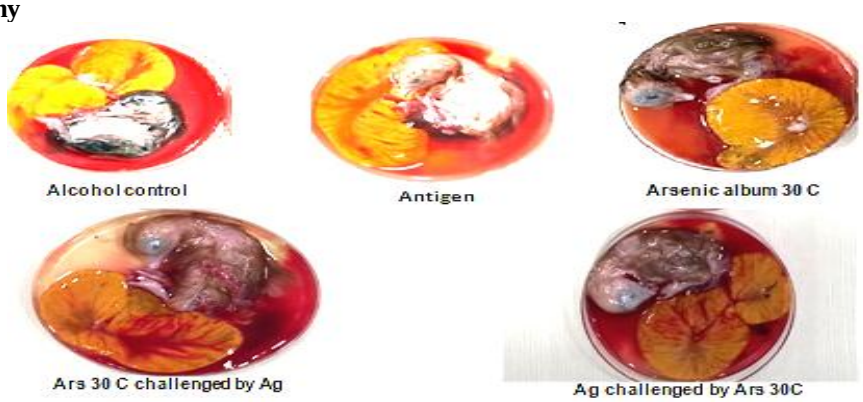

Fig. 5A: Morbid anatomy of embryos with pre and post treatment with *Arsenic* 30 C.

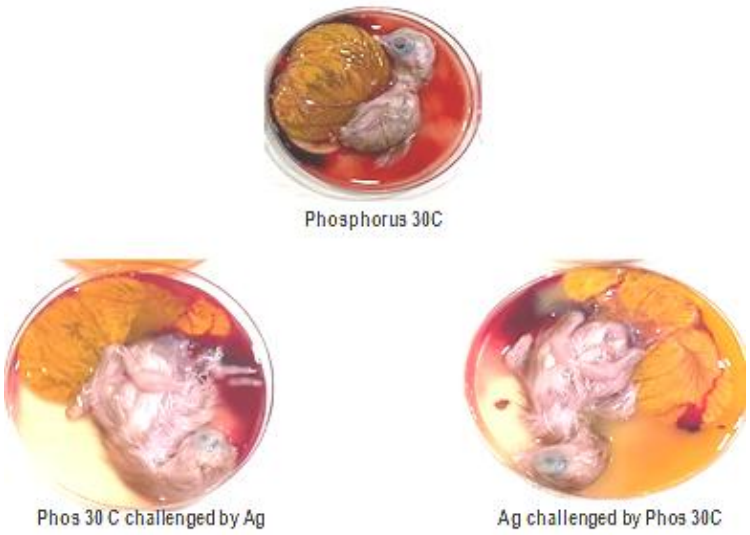

Fig. 5B: Morbid anatomy of embryos with pre and post treatment with *Phosphorus* 30 C.

Discussion

A recent study done by the clinicians which is a prospective parallel cluster cohort study, reported that individuals above 5 years of age received four pills of Arsenic album 30C and those who below 5 years received two pills of Arsenic album 30C [5]. The study population was 10,180 individuals living in 11 containment zones of Delhi, and among them 6590 were under alternative medicine intervention and 3590 individuals were under non-intervention cohort. The data was found to be quiet interesting as 74.40% (95% CI, 55.08 to 85.41) demonstrated protection with *Arsenic* 30C against the laboratory confirmed cases of COVID 19[5]. However, the researchers also mentioned that randomized control trials are also necessary to confirm the above findings[5]. In our previously reported findings on *Arsenic album* 6C as a pre and post treatment on spike protein induced pathological changes within fertilized chick egg model reported beneficial role of the medicine. In a clinical trial mentioned in the introduction part, carried out by Mukherjee, *et al*., 2022 reported that the group which received medicine Phosphorus 30CH had suffered the minimum from COVID-19 or the cases are mostly un-confirmed when compared with other two groups receiving *Bryonia alba* 30C and *Gelsemium sempervirens* 30C. These two medicines namely *Arsenic album* 30 C and *Phosphorus* 30C were reported to be able to give symptomatic relief in COVID 19 manifestations effectively as observed following the human trials[7].

The SARS-CoV-2 infection is primarily responsible for cytokine storm (CS) that causes collateral damage of the tissues all over the body[8]. The CS is usually associated with acute respiratory distress syndrome (ARDS) among human beings. CS systemic life damaging inflammatory syndrome is the cause of heightened amount of freely circulating cytokines and hyper activation of immune cells [8]. In case of infection with SARS-CoV, the infected dendritic cells (DC) expresses marginal levels of antiviral cytokine – Interferon (IFN) α, β, γ which in-turn up-regulates the expression of pro-inflammatory cytokines IL-6 and TNF-α[9, 10]. Other pro-inflammatory cytokines namely IL-1, IL-8, IL-12, TGF-β were observed to be much elevated among the critically infected patients in comparison to the non-critical ones[11 - 13]. When viruses infect human body, the pattern recognition receptors (PRRs) which are present upon the surface of the immune cells responsible for innate immunity can identify the exclusive molecular structures of the infecting virus which are collectively known as pathogen-associated molecular patterns (PAMPs). Thus, the PRRs and PAMPs stimulate the production of interferons and other cytokines through the activated signaling pathway. However, there occurs certain phenomenon within the body where several cytokines gets up-regulated within a short span of time resulting in CS[14-15]. In this study the

results indicated a good control of these cytokine surges by these medicines.

Interferons (IFNs)

Interferons (IFN) α, β, γ play a significant role in anti-viral response as the first line of defense by our immune system, and in turn, they inhibit the replication of viruses through several pathways. The two most important Interferons in terms of anti-viral activity are IFN α and γ[16]. The Interferons are secreted by the innate immune cells along with the virus-infected cells. For the researchers, the role of Interferons is a matter of interest as their roles in COVID-19 are considered to be controversial[17]. In our data, IFN α, the antigen itself could not induce the particular cytokine gene expression, whereas *Phosphorus* 30C could remarkably induce the gene expression of IFN α in both the preventive, curative sets and also in the *Phosphorus* 30C control set. When we observed the medicine control sets of *Arsenic* 30C, its preventive and curative sets, the gene expression got moderately increased in all the sets. Thus, it can be understood that *Phosphorus* 30C could induce a higher anti-viral response in comparison to *Arsenic* 30C, so this particular finding indicated the promising curative aspect of *Phosphorus* 30C than *Arsenic* 30C. IFN β got induced with the inoculation of antigen, and it was observed that with medicine *Phos* 30C alone, the gene expression level is much higher when compared with *Ars* 30C. In the curative sets of both Phos 30C and *Ars* 30C the IFN β gene expressions were found to be comparative. But in the preventive sets, it was observed that *Phos* 30C induced comparatively higher cytokine gene expression than *Ars* 30C. This finding also highlights the higher efficacy of *Phos* 30C than *Ars* 30C. In the case of IFN γ there were no such noticeable changes in the preventive and curative sets of medicine *Ars* 30C and *Phos* 30C. Although, medicine *Phos* 30C alone could stimulate the gene expression of IFN γ but with the addition of antigen it got suppressed.

Interleukins (IL -6, IL-10, IL-8, IL-1β)

Interleukin IL-6 is an inflammatory marker that has a direct association with COVID-19 disease progression. It is the most potential factor for the prediction of respiratory distress while the patient is suffering from the infection[18]. It also acts as a marker for hypoxia patients, those who would need oxygen therapy for treatment. It stimulates the JAK-STAT pathway responsible for the inflammatory action[18,19]. An elevated level of IL-6 was correlated with a higher level of complications among COVID-19 patients, as it enhances the blood pressure and the subsequent complications[20]. In the medicine control set *Phos* 30C had enhanced the gene expression of IL-6 when compared to *Ars* 30C. With *Ars* 30C, in the curative set, the expression of the IL-6 gene had decreased considerably. Another important finding was that there was considerably higher gene expression of IL-6 in all the experimental sets with *Phos* 30C which were in a controlled manner with medicine *Ars* 30C. This finding also highlighted the better efficacy of *Ars* 30C in terms of controlling the cytokine surges that cause detrimental effects on the health of the patients.

Another important cytokine is IL-10 which is considered to be a potent regulator of inflammatory response. It is considered to possess a multi-factorial role and prevents tissue damage by the immune system [21]. With the application of *Phos* 30C medicine, the gene expression of IL-10 was increased considerably in the medicine control set, in the curative set the changes in IL-10 gene expression were similar for both the medicines but in the preventive set *Ars* 30C could increase the expression significantly in comparison to *Phos* 30C. As IL-10 is considered to be a potent regulator of inflammatory response and prevents damage to the tissues, therefore, here *Ars* 30C holds promising preventive action. In the curative sets of both medicines, the changes were found to be similar.

IL-8 is a chemo-attractant cytokine produced by activated neutrophils in the inflammatory region. The cytokine possesses target specificity for neutrophils and not for any other blood cells[22]. In the preventive set of *Phos* 30C the gene expression of IL-8 had remarkably increased whereas no such noticeable change had been observed with the *Ars* 30C medicine. IL-8 was reported to be significantly higher in the serum among the morbid patients of COVID-19 compared to the

survivors. Therefore, it was correlated with the severity of the disease [23]. With the application of *Ars* 30C, the changes of IL-8 gene expressions were in control in all the experimental sets, therefore, *Ars* 30 showed better efficacy in controlling the severity of the disease.

Transforming Growth Factor (TGF) β1

TGF β genes are considered to be immunosuppressive factors that are responsible for noteworthy reduction of immunological functions by the process of reduction in recruitment of cells and in turn down-regulating the production of cytokines [24]. With the inoculation of antigen the gene expression was increased, however, both in the curative and preventive set of *Ars* 30C and *Phos* 30C, the increased TGF β gene had been down regulated. Thus ameliorative action is observed for both the medicine.

In our another research study on mechanism of action evaluation of *Phosphorus* 6C, we found that protective action of IL-10 was enhanced in the curative set, and the heightened expression of TGF β1 got ameliorated in the medicine control set [25]. In this experimental study our data depicts that *Phosphorus* 30C increases remarkably IFN α (anti-viral activity) and also directly increases IFN γ which is a non specific antimicrobial agent directly, thus highlighting its role in the curative action. *Arsenic* 30C increases IL-10 much more in comparison to *Phosphorus* 30C in the preventive set and controlled the abnormal surges of cytokines – IL-6 and IL-8.

Conclusion

Considering all these points we may claim that the curative action of *Phosphorus* 30C will be better than *Arsenic album* 30C but if we consider preventive action *Arsenic album* 30C is better than *Phosphorus* 30C.

Ethical Clearance

As the study was completed within 16th day cycle of embryonated chick eggs, therefore no ethical clearance was needed for the study. However, ratification was obtained from the Institutional Ethical Committee before the initiation of the research study on 22.07.2021.

Acknowledgement

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التأثير المقارن للأدوية البديلة ألبوم الزرنيخ CH٣٠ والفوسفور CH٣٠ لموازنة التعبيرات الجينية للسيتوكينات في التغيرات المرضية التي يسببها بروتين سبايك SARS-CoV-2

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قد تلعب الأدوية البديلة دورا مهما من حيث الإجراءات الوقائية والعلاجية ضد الأمراض الفيروسية مثل كوفيد-١٩. أظهرت بعض التجارب السريرية مع الأدوية البديلة ضد كوفيد-١٩ نتائج مثيرة للجدل. لذلك ، في هذه الدراسة ، استكشفنا فعالية اثنين من هذه الأدوية البديلة وهما ألبوم الزرنيخ ٣٠ Cوالفوسفور ٣٠ Cعلى نموذج بيض الفرخ المخصب (Gallus gallus domesticus) ضد التغيرات المرضية التي يسببها مستضد بروتين سبايك RBD (S1) ل.SARS- CoV- 2

في هذه الدراسة ، تم تحدي البويضات المخصبة البالغة من العمر ١٤ يوما ببروتين S1 جنبا إلى جنب مع الأدوية والضوابط المختلفة. بعد ٤٨ ساعة تم ملاحظة التشريح المرضي متبوعا بجمع السائل الألانتويك لمقايسة البيولوجيا الجزيئية. تمت دراسة التعبيرات الجينية السيتوكينية وهي إنترلوكينات - IL -6، IL-8، IL-10 ، إنترفيرون - IFN α، β، γ ؛ وعامل النمو التحويلي - TGF β1 بواسطة RT-PCR جنبا إلى جنب مع جين التدبير المنزلي β الأكتين. أشارت نتائج هذه الدراسة إلى دور مهم لهذه الأدوية ضد التغيرات المسببة للأمراض التي يسببها مستضد RBD S1 في نماذج البيض.