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Effect of Pinkwater BioSolve on expression of proinflammatory cytokines and histological changes in *Gallus domesticus* embryo

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ABSTRACT

Pinkwater Biosolve (BioSolve®) is one bioremediating chemical which has been widely used for cleanup of crude oil spill in Nigeria. It is a water-based formulation of nonionic surfactants and other specialty chemicals. The level of toxicity resulting from environmental exposure to this chemical has not been well understood.

The level of expression of proinflammatory cytokines and histological changes in *Gallus domesticus* embryo were investigated.

The embryo were pretreated with different doses of BioSolve, soil water from remediated soil sample, 10% soluble crude oil portion and a combination of the BioSolve with the soluble crude portion all constituted in normal saline solution. Reverse transcriptase PCR technique was used to assess the expression of hepatic proinflammatory cytokines. Histological examination was also carried out on liver fragments. The results showed that the pretreatment caused lesion on hepatocytes of all tested chick embryos except in the group administered with normal saline solution when compared with the normal control. The chick embryo exposed to 0.5 mg/kg BioSolve, 5% decanted soil water (v/v) obtained from crude oil remediated (using BioSolve) soil, and 10% (v/v) decanted crude oil remediated (using BioSolve) soil water all showed significant expression (at $p < 0.05$) of IFNγ, TGFβ1, IL-1β, IL-2 and TNF. The group treated with 10% soluble portion of crude oil showed significant changes in their expression pattern when compared with the control; TNF was up regulated, while IL-1β, IFNγ and TGFβ1 were down regulated. Only TNF was upregulated at $p < 0.05$ indicating the chances of soluble portion of crude oil causing cancer. IFNγ, TGFβ1, IL-1β and IL-2 were all down regulated significantly at $p < 0.05$ due to exposure to a combination of 10% soluble crude and 0.036 mg/kg BioSolve. The combination of 10% soluble crude and 0.36 mg/kg BioSolve caused lethal effect to the developing chick embryo.

1. Introduction

In Nigeria, the Department of Petroleum Resources (DPR) EGASPIN [1] has been the document used as the basis for assessing and certifying soil and water remediation after petroleum oil spill cleanup and remediation of the environment has been completed. The parameters generally considered from soil and water samples during the cleanup include: PH, total petroleum hydrocarbons, metals, and microbial activities [2]. Oil pollution and its cleanup processes involving chemicals has revealed that both dispersant alone and dispersant - oil mixtures may be more injurious to aquatic organisms than the oil alone [3].

Pinkwater BioSolve is one bioremediating chemical which has been widely used for cleanup of crude oil spill in Nigeria as DPR approved cleanup chemical agent [4,5]. Pinkwater Biosolve is a water-based formulation of nonionic surfactants and other specialty chemicals. It contains no caustic, hydrocarbon solvents, d-limonene or any other hazardous or restricted ingredients [6]. The exposure dose of BioSolve to humans in real life has not been documented. However, the LC50 for BioSolve from toxicity studies has been indicated as 7.2 ppm for *Menidia beryllina* after 96 h of exposure and 13.4 ppm for *Mysidopsis bahia* after 48 h of exposure [6].

Following the BP oil spill that occurred in 2010 at the Gulf of Mexico, Corexit dispersants were used for the cleanup of the oil spill. The reports further indicated that Corexit left the environment more toxic than the spill itself [7].Also studies by Mark, A. et al [8] showed that clean-up workers exposed to the oil spill and dispersant experienced significantly altered blood profiles, liver enzymes, and somatic symptoms.

It is therefore imperative to note that the exposure limits for the chemicals being used for cleanup of oil spills have not been classified in

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Table 1
Gene Specific Primer sequences.

GgIL1B_F	CTGCCTGCAGAAGAAGCCT
GgIL1B_R	TTGTAGCCCTTGATGCCCAG
Gg IL2_F	TGGAGCATCTCTATCATCAGAAAAA
Gg IL2_R	CCGGTGTGATTTAGACCCGTA
Gg IFNG_F	CTGGCCAAGCTCCCGATGAA
Gg IFNG_R	GAGCTGAGCAGGTATGAGTGG
Gg TGFB1_F	TGTACCAGGGTTACGGCAAT
Gg TGFB1_R	AACCCCCAAAAAGGGAACCAT
GgTNF_F	CCGCCCAGTTCAGATGAGTT
GgTNF_R	CCACACGACAGCCAAGTCAA
Gg ACTB_F	AATCAAGATCATTGCCCCACC
Gg ACTB_R	ATCCTGAGTCAAGCGCCAAA
Gg GAPDH_F	TTGACGTGCAGCAGGAACACT
Gg GAPDH_R	CGCTTAGCACCACCCTTCAG

F = FORWARD; R = REVERSE.

Nigeria.

Animals and humans from the oil rich Niger Delta obtain their food and water directly or indirectly from the cleaned up and remediated environments, thereby getting directly or indirectly exposed to the treatment chemicals such as Pinkwater BioSolve.

This study, therefore, assessed the effect of exposure to Pinkwater BioSolve on developing embryo in *Gallus domesticus*.

2. Materials and methods

2.1. Experimental design

All the test samples were constituted in normal saline solution and administered 0.1 ml dose per egg.

The eggs were grouped as follows:

1 Normal control- no administration of samples to the egg.
2 Normal saline– administered 0.1 ml dose per egg.
3 0.036 mg/kg BioSolve in normal saline.
4 0.36 mg/kg BioSolve in normal saline.
5 0.5 mg/kg BioSolve in normal saline.
6 10 % (v/v) decanted crude oil remediated (using BioSolve) soil water in normal saline.
7 5 % (v/v) decanted crude oil remediated (using BioSolve) soil water in normal saline.
8 10 % soluble portion of crude oil in normal saline.
9 Equal volumes of 10% soluble crude oil +0.036 mg/kg BioSolve –constituted in normal saline.
10 Equal volumes of 10% soluble crude oil +0.36 mg/kg BioSolve –constituted in normal saline.

2.2. Animal treatment

Fertile eggs of Marshall Breed of *Gallus domesticus* weighing between 50–55 g were obtained from the poultry farm at the Federal University of Agriculture, Abeokuta (FUNAAB) and certified fertile by the veterinarian in University of Benin, Nigeria.

The eggs were incubated for hatching in the laboratory incubator for 21 days.

Then the eggs were kept in an incubator (112 Digital Egg Incubator Machine with Automatic Hatchery Clear Egg Turning Temperature Control Farm Chicken Egg Incubator Controller) with temperature and relative humidity regulated at 37.4 °C and 60% respectively. The eggs were candled on day 4 to select the developing ones –which were returned to the incubator. On the eleventh day, the eggs (each) were inoculated with the test sample- each constituted in 0.1 ml of normal saline solution.

Eggs were candled again on day 8 to select only the growing ones. Inoculation was done on day 11.

To inoculate, the end of the egg where the air space is located was sterilized by cleaning with a swab of denatured alcohol. A pin hole was drilled in the center of the air cell with a sharp sterile needle a required amount of the test sample was dispensed onto the egg membrane, while care was taken not to penetrate the membrane. The egg was placed on the left hand and the needle directed into the pin hole horizontally.

After inoculation, the pin holes were sealed with paraffin wax and the eggs were placed undisturbed in a vertical position (with air space up) for re incubation. Control eggs were inoculated with normal saline alone.

Normal Saline (Ph 5.5–7.0)

Samples were all constituted in saline solution (0.1 ml dose).

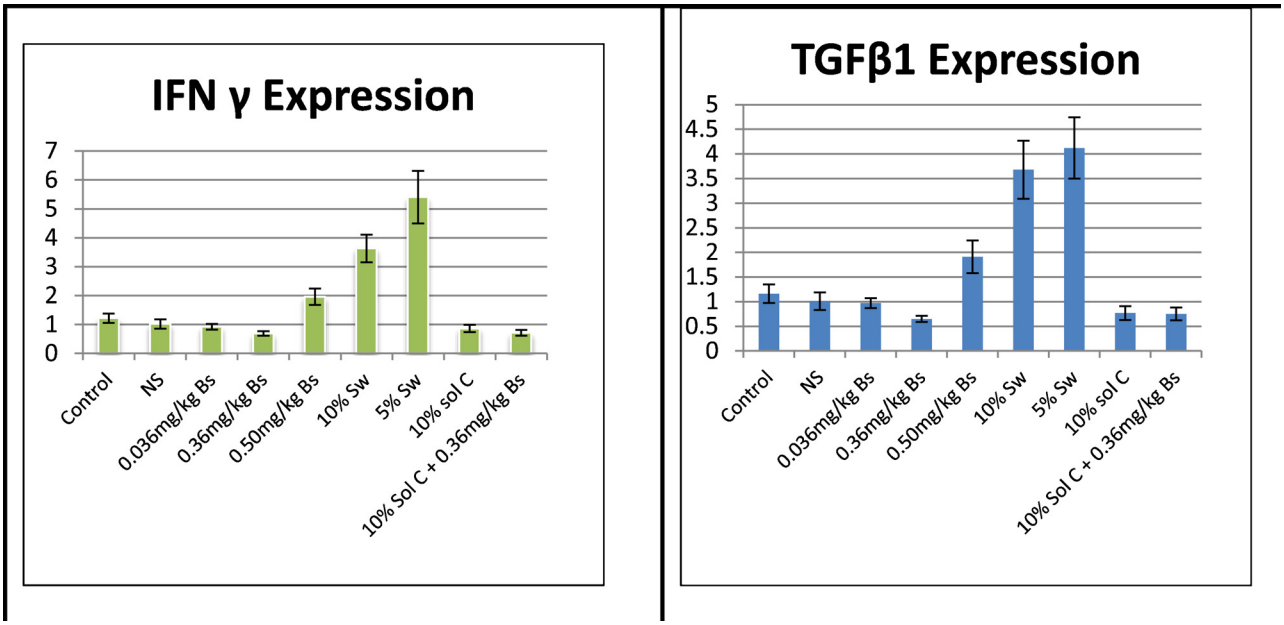


Fig. 1. Expression of proinflammatory cytokines with respect to the control group (NS = Normal Saline; Bs = BioSolve; 10% Sw = 10% remediated Soil water; 5% Bs = 5% remediated soil water; Sol C = Soluble crude portion).
IFN γ = interferon gamma; TGFβ1 = Transforming growth factor beta 1; IL-1β = Interkeukin 1 beta; IL-2 = Interleukin 2; TNF = Tumour necrosis factor.

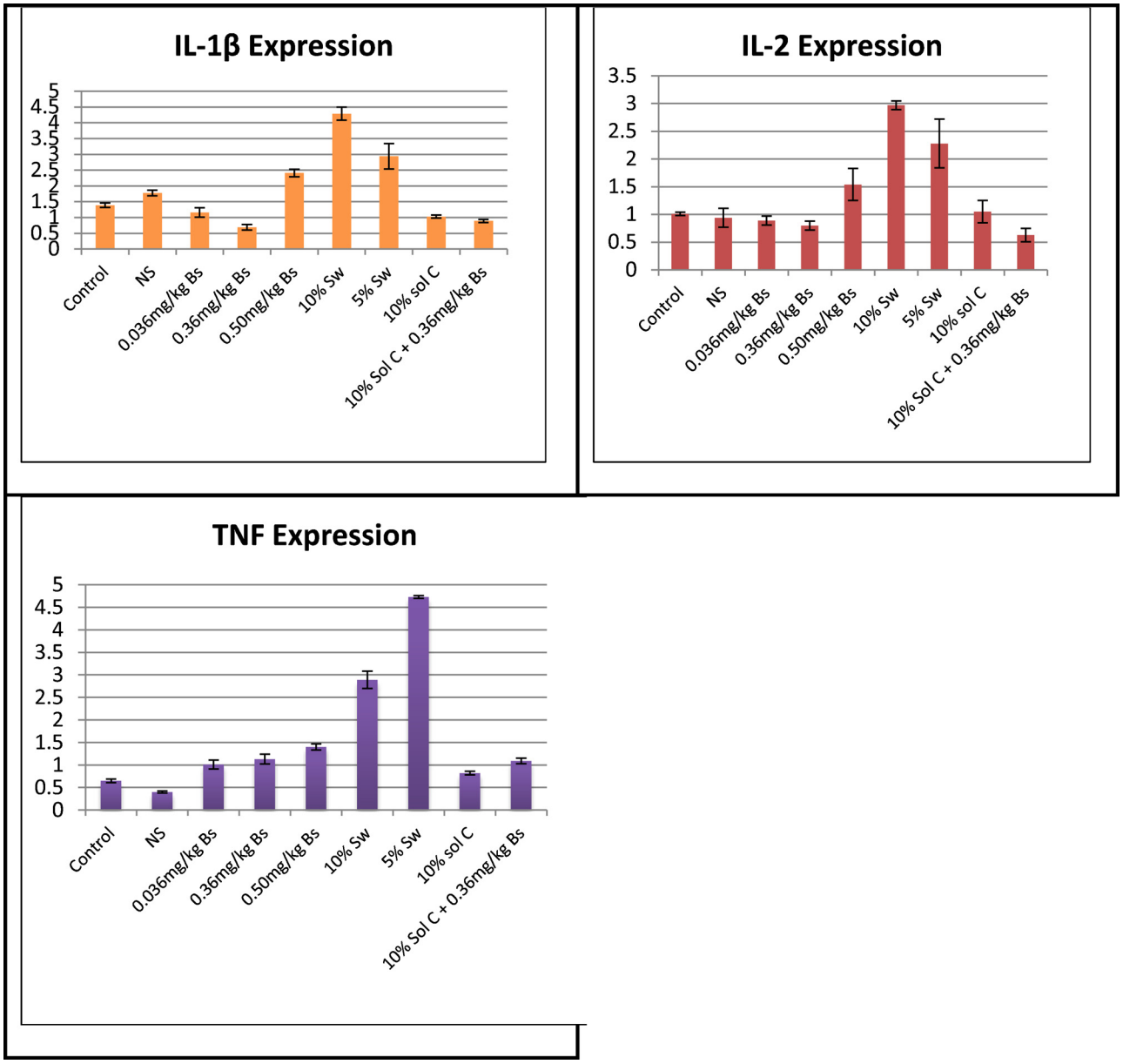


Fig. 1. (*continued*)

0.9% saline = 9 g/L NaCl.

2.3. Post innoculation management of eggs

On the 21 st day, after hatching, both live and moribund chicks were collected and placed according to their respective groups. Liver tissues of some live chicks and moribund chicks were immediately collected for histological examination and gene expression studies.

2.4. Expression of hepatic proinflammatory genes [9]

The levels of expression of certain hepatic proinflammatory genes were assessed using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) techniques. In brief, RNA from the liver samples was extracted using the spin column kit obtained from Aidlab'sEASYspinPlusVR (Aidlab Biotechnologies Co., Ltd, Beijing, China) according to the instructions of the manufacturer. The RT-PCR was carried out with 500 ng RNA template using the TransgenEasyScriptVR one-step RT-PCR supermix (Beijing TransGen Biotech Co., Ltd, Beijing, China) according to the instructions of the manufacturer. Samples were subjected to an initial incubation at 45 °C for 30 min for cDNA synthesis, followed by PCR amplification, using gene-specific primers (GSP) (Table 1), 94 °C for 5 min followed by 40 cycles of 94 °C for 30 s, 5 min at the annealing temperature of GSP, and 1 min at 72 °C. All amplifications were carried out in C1000 TouchTM Thermal Cycler (BioRad, Hercules, CA).

The intensity of the amplicon bands on 1.2% agarose was analyzed using Image J software [10]. Results were presented as the relative expression of the gene in comparison with the level of expression of b-actin gene.

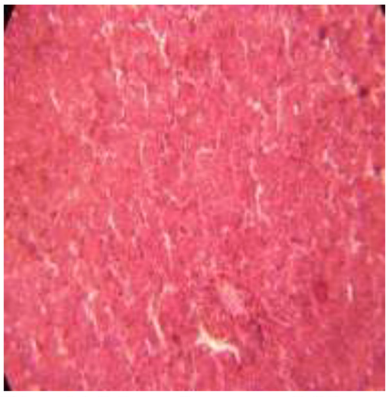
2.5. Histological study

The organs (Liver) were carefully removed and weighed individually and fixed in 10% (vol/ vol) formaldehyde, cleaned up in xylene and embedded in a paraffin wax (melting point at 56 percent). Tissue sections were prepared according to the method of Drury and Wallinton [11] and stained with eosin/ hematoxylin. Photomicrographs were taken at ×400 using a digital camera.

A

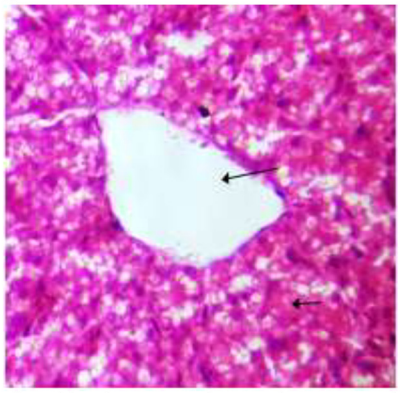
Liver histology shows no visible lesion (x400)

B



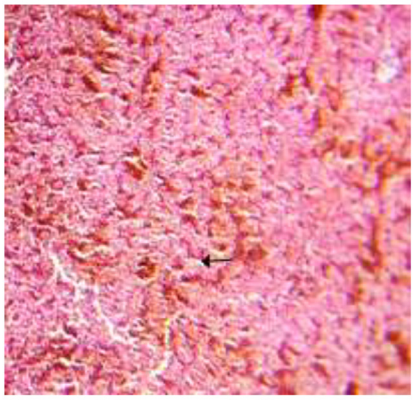
Liver histology shows no visible lesion (x400)

C



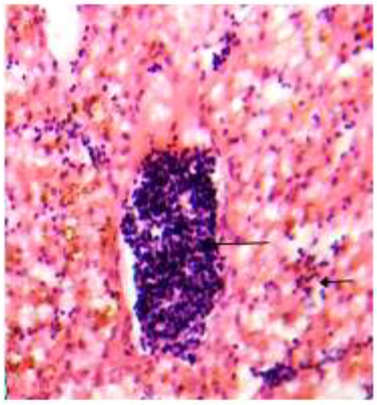
Liver histology reveals prominent central vein (long arrow) with visible hepatocytes with pyknotic nucleus (short arrow) (x400)

D



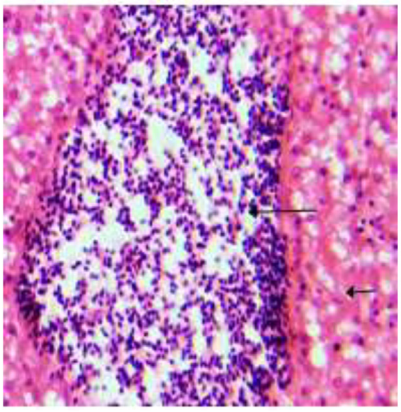
Liver histology reveals distorted hepatocytes with fibrotic changes (short arrow) (x400)

E



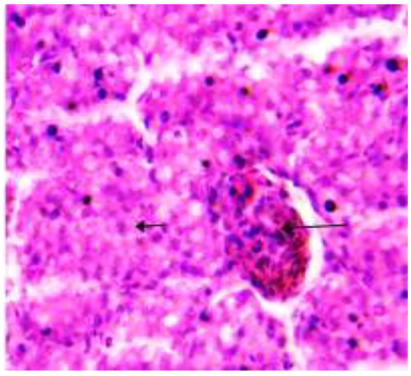
Liver histology reveals congested central vein (long arrow) with dispersed inflammatory cells, there is mild fatty changes too (short arrow) (x400)

F



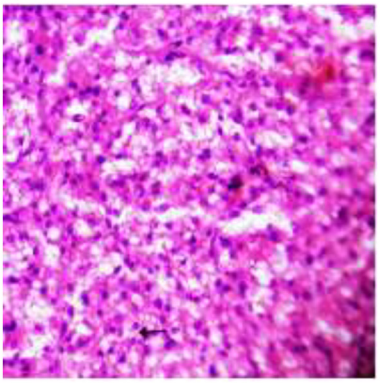
Liver histology reveals congested central vein (long arrow) with visible inflammatory cells, and mild fatty changes (short arrow) (x400)

G



Liver histology reveals congested central vein (long arrow) with visible hepatocytes, there is mild fatty changes too (short arrow) (x400)

H



Liver histology reveals with visible hepatocytes with pyknotic nucleus (long arrow), there is mild fatty changes (short arrow) (x400)

(caption on next page)

Fig. 2. Photomicrographs of liver from day old chicks given the following treatments:
A. Normal control- no administration of samples to the egg.
B. Normal saline– administered 0.1 ml dose per egg.
C. 0.036mg/kg BioSolve in normal saline.
D. 0.36mg/kg BioSolve in normal saline.
E. 10% (v/v) decanted crude oil remediated (using BioSolve) soil water in normal saline.
F. 5% (v/v) decanted crude oil remediated (using BioSolve) soil water in normal saline.
G. 10% soluble crude oil portion in normal saline.
H. Equal volumes of 10% soluble crude oil portion + 0.036 mg/kg BioSolve –both constituted in normal saline.

2.6. Statistics

Data were expressed as mean ± standard deviation of three replicates in each group. Student's *t*-test was used to test for level of significance at $p < 0.05$ among the groups.

3. Results and discussion

The gene expression pattern of the experimental groups is as presented in Fig. 1 below:

The result above shows the expression of the proinflammatory cytokines. The tested cytokines were interferon gamma (IFN γ), Transforming growth factor beta 1(TGFβ1), Interkeukin 1 beta (IL-1β), Interleukin 2 (IL-2) and Tumour necrosis factor (TNF).

The gene expression results of experimental groups were compared with that of the control group. From the result, the proinflammatory cytokines in treatment groups with values higher than that of the control group indicate an 'up regulation' of the cytokines, while lower values to that of control indicate 'down regulation' or suppression of the cytokines.

Furthermore, the chick embryos exposed to 0.5 mg/kg BioSolve, 5% decanted soil water(v/v) obtained from crude oil remediated (using BioSolve) soil, and 10% (v/v) decanted crude oil remediated (using BioSolve) soil water respectively, all showed significant expression (at $p < 0.05$) of the five proinflammatory cytokines tested [i.e. interferon gamma (IFN γ), Transforming growth factor beta 1(TGFβ1), Interkeukin 1 beta (IL-1β), Interleukin 2 (IL-2) and Tumour necrosis factor (TNF)] when compared to the normal control.

Cytokines are secreted, small molecules that modulate the immune response and have an essential role during T cell differentiation. It was believed previously that each cytokine exerts immune stimulatory (inflammatory) or inhibitory (anti-inflammatory or regulatory) activities [12]. As further reviewed by Shachar and Karin [12], diseases in which the immune system attacks and damages self-components are categorized as autoimmune or autoinflammatory diseases. In autoimmune diseases, cells of a specific arm of the immune system direct the pathogenesis.

Romagnani [13] reported that Type 1 T helper (Th1) cells produce interferon-gamma, interleukin (IL)-2, and tumour necrosis factor (TNF)-beta, which activate macrophages and are responsible for cell-mediated immunity and phagocyte-dependent protective responses. Th1 cells are involved in the pathogenesis of organ-specific autoimmune disorders, Crohn's disease, Helicobacter pylori-induced peptic ulcer, acute kidney allograft rejection, and unexplained recurrent abortions. This suggests that the survival rate of the newly born chicks would be low.

Dinarello [14] also reviewed that some cytokines act to make disease worse (proinflammatory), whereas others serve to reduce inflammation and promote healing (anti-inflammatory). Interleukin (IL)-1 and tumor necrosis factor (TNF) are proinflammatory cytokines, and when they are administered to humans, they produce fever, inflammation, tissue destruction, and, in some cases, shock and death.

Also Cytokines with a proinflammatory function are elevated in the infected tissue. Over time, some of them increase systemically [15,16].

The group treated with 10% soluble portion of crude oil showed significant changes in their expression pattern when compared with the control; TNF was up regulated, while IL-1β, IFN γ and TGFβ1 were down regulated.

Only TNF was upregulated at $p < 0.05$, indicating the chances of soluble portion of crude causing cancer. IFN gamma, TGF beta 1, IL-1 beta and IL-2 were all down regulated significantly at $p < 0.05$ due to exposure to a combination of 10% soluble crude and 0.036 mg/kg BioSolve.

The combination of 10% soluble crude and 0.36 mg/kg BioSolve caused death to the developing chick embryo.

It is imperative to note that IL-1β in the normal saline group showed a significant increase when compared with the normal control thus indicating a sign of pain or inflammation which may have been caused by the use of normal saline as a medium of treatment. IL-1β is a proinflammatory cytokine that has been implicated in pain, inflammation and autoimmune conditions [17].

A closer look at the histopathology in Fig. 2 below further explains the phenotypic changes that occurred in the chick embryo due to exposure to the test components.

4. Conclusion

From the result, Pink water BioSolve found in underground/surface water was toxic to developing chick embryo in the concentration as low as 0.036 mg/kg BioSolve. 10% soluble crude oil portion was also toxic to the chick embryo both alone and in combination with the BioSolve.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.toxrep.2020.04.009.

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