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Effect of Pink Water Biosolve on Expression of some Cytokines in Hepatocyte of Wistar Rat

Abstract

The use of Pink Water Biosolve for clean-up of the environment as a bioremediating chemical has a wide application in the clean-up of crude oil spill in Nigeria. BioSolve is a water-based formulation of nonionic surfactants and other specialty chemicals. The level of toxicity resulting from environmental exposure of plants and animals, including humans, to this chemical has not been well understood. Thus, the level of expression of pro-inflammatory and anti-inflammatory cytokines in liver cells of Wistar rats exposed to drinking water obtained from cleaned-up environment was investigated in this study. Reverse transcriptase PCR technique was used to assess the expression of selected hepatic cytokines. The results showed that 10% Soluble Crude Oil Portion (SCP) caused an increased expression of the Interleukin 1-beta (IL-1β), Nuclear Factor kappa-B (NF-κB), Interleukin 6 (IL-6), Interleukin 10 (IL-10), Mitogen-activated protein kinase 8 (MAPK8) and Toll-like receptor 4 (TLR4) respectively when compared to the control. The combination of 10% SCP with 0.36mg/L BS and 0.50mg/L BS respectively caused significant ($p<0.05$) increases in the expression of IL-6, IL-10, MAPK8 and TLR4 respectively. IL-1B was non-significantly ($p<0.05$) increased when compared to the control. This suggests that the presence of the substance in underground/surface water, if found in concentration as low as 0.36mg/L, would be toxic to Wistar rats when consumed over a prolonged period (above 2 weeks).

Keywords

BioSolve • Pro-inflammatory cytokines • Anti-inflammatory cytokines • Water • Clean-up • Remediation

Research Article

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Introduction

Oil dispersants (chemical agents such as surfactants, solvents, and other compounds) are used to reduce the effect of oil spills by changing the chemical and physical properties of the oil [1]. Pink Water biosolve also known as “BioSolve” is a water-based formulation of nonionic surfactants and other specialty chemicals has been widely used in Nigeria as a bioremediating chemical for clean-up of crude oil spill [2]. The Safety Data Sheet of the chemical shows that it contains no caustic, hydrocarbon solvents, d-limonene or any other hazardous or restricted ingredients [3].

Cytokines, on the other hand, are produced by cells which are not organized in special glands, and which act systemically to affect biological phenomena such as inflammation, wound healing, organogenesis and oncogenesis. Inflammatory cytokines such as the interleukins and interferons, growth factors such as epidermal and hepatocyte growth factor and chemokines, are critical controllers of cell, and hence tissue, growth, migration, development and differentiation [4]. Cytokines may be “good” when stimulating the immune system to fight a foreign pathogen or attack tumours or may be “bad” when their expression causes inflammatory diseases, such as the role of tumour necrosis factor α in rheumatoid arthritis or asthma and Crohn’s disease [5].

Over the past 2 decades, the Niger Delta axis of Nigeria has witnessed a wide use of the bioSolve for clean-up of oil polluted environments, as well as its application for tank cleaning activities in the oil and gas industries [2]. These cleanup activities introduce varying quantities of the chemical into our drinking water bodies, thereby contaminating the waters in varying degree [1 6]. The dangers associated with such exposures have not been well researched in Nigeria. However, animals and humans from the oil rich Niger Delta obtain food and water directly or indirectly from the cleaned up and remediated environments, thereby getting directly or indirectly exposed to the treatment chemicals like Pink Water biosolve used in the cleanup of the environment. This study therefore assesses the effect the exposure of Wistar Rats to Pink Water biosolve would have on the expression of some hepatic cytokines in the animal.

Materials and Methods

Animal Treatment

Male Wistar albino rats of six to seven weeks old (120-130g) were used for this study. Throughout the period of experiment, the rats were housed in plastic breeding cages containing feed and water, with wire gauze on the lids as source of ventilation. Good hygiene was maintained during the study in the various cages as wood shavings which were used as bedding material were being changed every 2 days.

Diets

Throughout the period of the study including the first two weeks of acclimatization, the rats were fed with standard pelleted feed purchased from Kenegod Services, Benin City, Edo state.

Preparation of Soluble Fraction of Crude Oil (SCP)

The crude oil (Escravos Light) was used for this study. The soluble fraction was isolated by mixing crude oil with distilled water in a 1:2 ratio (that is 500ml of crude oil to 1000ml of distilled water) in a glass conical flask and stirring for 8-24 hours using a magnetic stirrer. After stirring, the mixture was poured into a separating funnel and left to fractionate for 15-20 hours the soluble portion was then collected. The clear portion of the separation was decanted out as soluble fraction of crude oil (SCP).

Administration of Samples to the Rats

Samples were administered using oral gavage, the rats were weighed and were given 0.50 ml of samples every day for 30 days as shown in (Table 1) below. The samples were stored under room temperature throughout the duration.

Note

1. All the animals were fed with the normal feed pellets all through the experimental period.
2. A mixture of equal volumes of 10% SCP and varying milligrams per kilogram (0.036mg/L. 0.36mg/L and 0.5mg/L) of bioSolve represent possible bye-products of interaction between soluble portion of crude oil and the biosolve used in the clean-up of the environment.
3. BS = BioSolve (Pink Water biosolve).

Collection of tissue samples

Upon completion of administration phase, the rats from each group were allowed to fast overnight and sacrificed after they were anaesthetized with diethyl ether. Liver organs were harvested into trizol solution in vial for gene expression studies.

Expression of Hepatic Proinflammatory Genes

Expression of hepatic proinflammatory genes. The levels of expression of certain hepatic proinflammatory genes were assessed using semi-quantitative reverse transcriptase polymerase chain reaction (RT-PCR) techniques as described. [7]. In brief, RNA from the liver samples was extracted using the spin column kit obtained from Aidlab's EASYspin PlusVR (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 Transgen EasyScriptVR 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 45oC for 30 min for cDNA synthesis, followed by PCR amplification, using gene-specific primers (GSP) (Table 1), 94oC for 5 min followed by 40 cycles of 94oC for 30s, 5 min at the annealing temperature of GSP, and 1 min at 72oC. 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 [8]. Results were presented as the relative expression of the gene in comparison with the level of expression of housekeeping gene glyceraldehydes3-phosphate dehydrogenase (GAPDH). (Table 2)

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.

Experimental group	Treatment received (administered 0.5ml dose daily)
Control	No sample received
0.036mg/L BS	0.036mg/L Biosolve
0.36mg/L BS	0.36mg/L Biosolve
0.50mg/L BS	0.50mg/L Biosolve
10% SCP	Soluble portion of crude oil (10%)
10% SCP + 0.036mg/L BS	Equal volumes of 10% soluble crude oil + 0.036mg/L Biosolve
10% SCP + 0.36mg/L BS	Equal volumes of 10% soluble crude oil + 0.36mg/L Biosolve
10% SCP + 0.50mg/L BS	Equal volumes of 10% soluble crude oil + 0.50mg/L Biosolve

Table 1. Administration of samples to wistar rats.

Gene	Forward Sequence (5' -3')	Reverse sequence
TLR4	TATCCAGGTGTGAAATTGAGACA	AAGGCTTGGGCTTGAATGGA
NF-κB1	TCCCACAAGGGGACATTAAGC	CAATGGCCTCTGTGTAGCCC
MAPK8	TCAGCCGGCCATTTCAGAAT	GTTGATGTACGGGTGCTGGA
IL-1β	CCTTGTGCAAGTGTCTGAAGC	TCAGACAGCACGAGGCATTT
IL-6	TCCGGAGAGGAGACTTCACA	GAATTGCCATTGCACAACTCTT
IL-10	TGCGACGCTGTCATCGATTT	GTAGATGCCGGGTGGTTCAA
Gg GAPDH	TTGACGTGCAGCAGGAACACT	CGCTTAGCACCACCCTTCAG

IL-1β= Interleukin 1 beta; IL-6 = Interleukin 6; IL-10 = Interleukin 10; MAPK8 = Mitogen-activated protein kinase 8; NF-κB = Nuclear Factor kappa-light-chain-enhancer of activated B cells; TLR4 =Toll-like receptor 4. To normalize mRNA expression, the expression of the housekeeping gene glyceraldehydes3-phosphate dehydrogenase (GAPDH) was measured for comparative reference.

Table 2. Gene specific primer sequences for wistar rats.

Results and Discussion

Following the Use of Pink Water biosolve for Crude Oil Spill Remediation. (Table 3) From the result above, increasing doses of bioSolve caused significant ($p<0.05$) expression or increases of IL-1B, IL-10, MAPK8 respectively when compared with the control. TLR4 was also increasingly expressed with increasing level of the bioSolve while IL-6 was increased (though not consistently increased with increasing doses of bioSolve) when compared with the control. 0.36mg/L BS caused a significant ($p<0.05$) expression of NF-kB in wistar rats when compared with the control. In a review [9], the activation of NF-*κ*B is said to result in the production of proinflammatory mediators such as TNF, IL-6, and IL-1*β*. This subclass of cytokines is referred to as "proinflammatory cytokines" due to their ability to promote inflammation in response to tissue injury and infection.

The result in the table 2 above also shows that 10% SCP caused non-significant ($p<0.05$) increases in the expression of the cytokines IL-1B and NF-kB while significantly ($p<0.05$) increasing the expression of IL-6, IL-10, MAPK8 and TRL4 respectively when compared to the control. IL-10 has been identified as anti-inflammatory cytokine which has been well researched in the pathogenesis of Inflammatory Bowel Disease (IBD) [10,11]. IL-10 controls inflammatory processes by suppressing the expression of proinflammatory cytokines, chemokines, adhesion molecules, as well as antigen-presenting and costimulatory molecules in monocytes/macrophages, neutrophils, and T cells. The review [9] further shows that early *in vitro* studies demonstrated IL-10 suppresses monocytes/macrophage-derived proinflammatory cytokines such as TNF-*α*, IL-1, IL-6, IL-8, and IL-12.

Upregulation of stress response genes including *NFκB,* in turn upregulate the production of proinflammatory cytokines such as interleukin-1*β* (IL-1*β*), Interleukin-6 (IL-6), and tumour necrosis factor-*α* (TNF-*α*). These proinflammatory cytokines are responsible for initiating inflammation in response to tissue injury [9,12]. However, NF-*κ*B1 is a member of the ubiquitous transcription factor that collectively comprises of the following five members: NF-*κ*B1 (p50/p105), NF-*κ*B2 (p52/p100), p65 (Rel A), Rel 3, and cRel [13]. MAPK families play an important role in complex cellular programs like proliferation, differentiation, development, transformation, and apoptosis [14]. JNK1, also known as MAPK8, is expressed in most tissues and is involved in transduction of extracellular signals such as growth factors or cytokines through a phosphorylation cascade to elicit diverse intracellular responses [15].

The result in table 2 also shows that the combination of 10% SCP with 0.36mg/L BS and 0.50mg/kg BS respectively caused significant ($p<0.05$) increases in the expression of IL-6, IL-10, MAPK8 and TLR4 respectively. IL-1B was non-significantly increased when compared to the control. Recall that NF*κ*B acts to induce gene expression of many cytokines involved predominantly in mucosal inflammation, and angiogenesis, chemokines, immunoreceptors, cell adhesion molecules, proapoptotic and antiapoptotic as well

Treatment groups	IL-1β	IL-6	IL-10	MAPK8	NF-κB	TLR4
Control	0.58 ± 0.04	0.59 ± 0.01	0.80 ± 0.02	0.62 ± 0.02	0.75 ± 0.08	0.91 ± 0.05
0.036mg/L BS	0.91 ± 0.02[a]	0.73 ± 0.07	0.44 ± 0.03[a]	1.34 ± 0.06[a]	0.91 ± 0.05	1.06 ± 0.09
0.36mg/L BS	1.16 ± 0.03[a]	0.92 ± 0.04[a]	1.19 ± 0.12[a]	1.18 ± 0.07[a]	1.06 ± 0.01[a]	1.13 ± 0.14
0.50mg/L BS	1.29 ± 0.18[a]	0.82 ± 0.10	1.60 ± 0.05[a]	1.59 ± 0.02[a]	0.68 ± 0.11	1.85 ± 0.10[a]
10% SCP	0.65 ± 0.02	0.77 ± 0.07[a]	0.97 ± 0.01[a]	1.26 ± 0.11[a]	0.77 ± 0.02	1.18 ± 0.08[a]
10% SCP + 0.036mg/L BS	0.96 ± 0.14	1.03 ± 0.05[a]	0.98 ± 0.06	0.91 ± 0.04[a]	0.18 ± 0.01[a]	0.82 ± 0.02
10% SCP + 0.36mg/L BS	0.60 ±0.06	0.52 ± 0.03[a]	1.29 ± 0.03[a]	1.30 ± 0.05[a]	0.27 ± 0.02[a]	3.69 ± 0.15[a]
10% SCP + 0.50mg/L BS	0.81 ± 0.01 [a]	0.96 ± 0.11[a]	0.98 ± 0.09	1.08 ± 0.12[a]	0.65 ± 0.08	2.36 ± 0.16[a]

Results presented as Mean ± SEM, n=3. "a" means values significant at $p<0.05$; df=4; **BS** means BioSolve, **SCP**=Soluble Crude Portion **IL-1β**= Interleukin 1 beta; **IL-6** = Interleukin 6; **IL-10** = Interleukin 10; **MAPK8** = Mitogen-activated protein kinase 8; **NF-κB** = Nuclear Factor kappa-light-chain-enhancer of activated B cells; **TLR4** =Toll-like receptor 4.

Table 3. Expression of cytokines in the liver of wistar rats following 30 days of administration of experimental samples.

as stress response genes. Only NF-kB was significantly ($p<0.05$) reduced by the consumption of the mixtures. This suggests that the increases observed in the expression of the proinflammatory cytokines (IL-1B and IL-6) in the result may be due to a mechanism other than the NF-kB induction pathway [9]. Defects in JNK signaling have been observed in inflammatory and neurodegenerative disorders [15]. For example, increased JNK1 activity leads to hyperphosphorylation of tau in Alzheimer's disease [16]. Amongst them, c-Jun N-terminal kinases (JNKs) are activated in AD brains and are also associated with the development of amyloid plaques.

Increasing evidence indicates that Toll-like receptor 4 (TLR4) plays a key role in the development of sepsis. Cell death is also thought to contribute to septic brain injury Modulation of TLR4 may result in the regulation of neuron cell death pathways by regulating autophagy in cortical tissues. For instance, septic brain injury induction by cecum ligation and puncture evoked autophagy have been associated with increased TLR4 expression [17]. Collective findings from a wide range of cytokine investigations indicate that the net effect of the inflammatory response is determined by a delicate balance between pro- and anti-inflammatory cytokines. Perturbations in this equilibrium can drive the host defence immune response either towards chronic inflammation or towards healing [10].

Conclusion

From the result, Pink Water bioSolve caused increased expression in all the hepatic cytokines tested. This suggests that the presence of the substance in underground/surface water, if found in concentration as low as 0.36mg/L, is toxic to Wistar rats when consumed over a prolonged period. This result can be extrapolated to that of humans. Thus, clean-up and remediation of crude oil polluted environment involving application of the bioSolve need to be extensively monitored to ensure proper application and that filtration of noxious substances into the household drinking water is prevented.

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Conflict of Interest

None to report.

References

1. National Research Council. "Oil Spill Dispersants: Efficacy and Effects". 2005
2. Sogbanmu, T. O, and A. A. Otitoloju. "Efficacy and Bioremediation Enhancement Potential of Four Dispersants Approved for Oil Spill Control in Nigeria." *J. Bioremed Biodegrad* 3 (2012): 1-5
3. Edet, Francis, Stella Olubodun, Sylvester Uansoje and George Eriyamremu et al. "Effect of Pinkwater Biosolve on Expression of Proinflammatory Cytokines and Histological Changes in Gallus Domesticus Embryo." *Toxicol Rep* 7 (2020): 1634-1639
4. Foster, John R. "The Functions of Cytokines and Their Uses in Toxicology." *Int. J. Exp. Pathol.* 82 (2001): 171-192
5. Ramani, Thulasi, Carol S. Auletta and Gregory Bannish et al. "Cytokines: The Good, The Bad, And the Deadly." *IJT* (2015): 355-365
6. Veľková, Veronika, Helena Hybská, and Tatiana Bubeníková. "Possible Oil Spills Disposal for Environmental Water-Body Protection." In Recent Oil Spill Challenges That Require More Attention. IntechOpen, 2022

7. Rotimi, Solomon Oladapo, Goodness Esther Bankole, Isaacson Bababode Adelani, and Oluwakemi Anuoluwapo Rotimi. "Hesperidin Prevents Lipopolysaccharide-Induced Endotoxicity in Rats." *Immunopharmacol Immunotoxicol.* 38 (2016): 364-371

8. Abràmoff, Michael D, Paulo J. Magalhães, and Sunanda J. Ram. "Image Processing with Imagej." *J Biophotonics* 11 (2004): 36-42

9. Sultani, Masooma, Andrea M. Stringer, Joanne M. Bowen, and Rachel J. Gibson. "Anti-Inflammatory Cytokines: Important Immunoregulatory Factors Contributing to Chemotherapy-Induced Gastrointestinal Mucositis." *IJCP* 2012 (2012)

10. Opal, Steven M, and Vera A. DePalo. "Anti-Inflammatory Cytokines." *Chest* 117 (2000): 1162-1172

11. Asadullah, Kh, W. Sterry, and H. D. Volk. "Interleukin-10 Therapy—Review of A New Approach." *Pharmacol Rev* 55 (2003): 241-269

12. Logan, Richard M., Rachel J. Gibson, Stephen T. Sonis, and Dorothy MK Keefe. "Nuclear Factor-Kb (Nf-Kb) And Cyclooxygenase-2 (Cox-2) Expression in The Oral Mucosa Following Cancer Chemotherapy." *Oral Oncol* 43 (2007): 395-401

13. Catz, Sergio D, and Jennifer L. Johnson. "Transcriptional Regulation of Bcl-2 By Nuclear Factor Kb And Its Significance in Prostate Cancer." *Oncogene* 20 (2001): 7342-7351

14. Zhang, Wei, and Hui Tu Liu. "Mapk Signal Pathways in The Regulation of Cell Proliferation in Mammalian Cells." *Cell Res* (2002): 9-18

15. Bubici, Concetta, and Salvatore Papa. "Jnk Signalling in Cancer: In Need of New, Smarter Therapeutic Targets." *Br J Pharmacol.* 171 (2014): 24-37

16. Ploia, Cristina, Xanthi Antoniou, Alessandra Sclip and Nadia Canu, et al. "Jnk Plays a Key Role in Tau Hyperphosphorylation in Alzheimer's Disease Models." *Am J Alzheimer's Dis* 26 (2011): 315-329

17. Li, Yafei, Li Zhang, Jun Tang and Fengyan Zhao, et al. "Role of Toll-Like Receptor 4 In the Regulation of The Cell Death Pathway and Neuroinflammation." *BRB* 148 (2019): 79-90

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