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In vitro investigations on the effects of semi-synthetic, sulphated carbohydrates on the immune status of cultured common carp (*Cyprinus carpio*) leucocytes

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1 ***In vitro* investigations on the effects of semi-synthetic, sulphated carbohydrates on the**
2 **immune status of cultured common carp (*Cyprinus carpio*) leucocytes.**

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16 Key words: Immunostimulants; Beta-glucan; Carbohydrates modification; Immune
17 responses; Innate immunity; Cytokines; *Cyprinus carpio*.

18
19 **Highlights**

- 20
21 • Modification to methyl hydroxyethyl cellulose produced a novel carbohydrate
22 (MHCS).
23 • Modified glucan (MHCS) exhibited consistent biological activities.
24 • MHCS induced strong respiratory burst in leucocytes without impacting viability.
25 • MHCS induce the expression of inflammation-related genes in carp immune cells.
26 • MHCS enhanced the effect of Poly I:C on carp leucocytes.

27
28 **Abstract**

29
30 The rapid emergence of drug resistance, unfavourable immunosuppression and mounting
31 evidence to suggest the deleterious accumulation of drug breakdown residues within animal
32 tissues has driven a strong desire to move away from these current methods of disease
33 control. Some natural products such as β -glucan, which are extracted from, for example,
34 plants and fungi, are able to modulate the immune system and increase protection against
35 diseases. However, these products are heterogeneous and their effects can be variable thus
36 limiting their applicability and reliability. Carbohydrates were modified via chemical
37 sulphation and these semi-synthetic, sulphated carbohydrates analysed for their
38 immunological activity utilising carp pronephric cells and a carp leucocyte cell line (CLC). A
39 sulphated $\beta(1,4)$ -glucan, methyl hydroxyethyl cellulose sulphate (MHCS), demonstrated a
40 stimulatory effect on fish immune cells. MHCS induced a range of bioactive effects in carp
41 leucocyte cells whilst not affecting cell viability when cells were exposed for 24h at
42 concentrations of 1-150 μgml^{-1} . MHCS stimulated the innate immune system where a
43 significant increase in respiratory burst activity was observed at concentrations 25-250 μgml^{-1}
44 in comparison to control (sterile water), cellulose ether, MacroGard[®] and zymosan. Also,
45 under in mock bacterial and viral infection conditions i.e. Lipopolysaccharide (LPS) and
46 polyinosinic:polycytidylic acid (Poly(I:C)), MHCS enhanced the immune responses of
47 pronephric cells by stimulating the respiratory burst activity at concentrations 50 and 150
48 μgml^{-1} . MHCS also enhanced the expression of cytokines including interleukin 1 beta (IL1 β),

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