

## Effect of Ganoderma Lucidum, Astaxanthin, Liv.52HB and STC30 on C - Reactive Protein Concentrations of Animal Model with CCL4 Induced Hepatocellular Carcinoma

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### ABSTRACT

Cancer is a major cause of death in developed countries and second in developing countries. Primarily, hepatocellular carcinoma (HCC) represents 4% of all types of new cases of cancer diagnosed globally and hence making it the most frequently diagnosed globally. This research studied the effect of some natural products (Ayurvedic) commonly used in the management of hepatitis and HCC on serum C-Reactive Protein (CRP) concentration of animal with CCL4 induced hepatocellular carcinoma with the aid of providing and documenting information on some natural products which have been believed to be effective for the management of HCC using C-reactive protein as a marker. Albino rats of Wistar strain with weight range of 60-120g were used for this study. The animals were divided into seven (7) groups of five (5) rats each. Group 1 was the normal control and was not induced while animals in group 2 to 7 where all induced for HCC using CCL4. Group 2 served as positive control and where not treated while animals in group 3 to 7 received various form naturopathic treatment; Ganoderma lucidium, Astaxarthin, Liv52 HB and STC30 respectively. The treatment lasted for a period of three weeks (21 days). Twelve (12) hours after the last treatment, the animal where sacrifice under chloroform anesthesia and blood samples collected via cardiac puncture. The blood sample were allowed to clot by standing then centrifuged at 4000rpm for 10 minutes to separate the blood cells from serum. The serum was separated and used for C - reactive protein concentration estimation. Result of analysis showed the C-Reactive Protein concentration of all induced groups; groups 2 or positive control ( $584.20 \pm 11.08$ ), group 3 or Ganoderma treated group ( $491.40 \pm 26.80$ ), group 4 or Astaxarthin treated group ( $378.00 \pm 37.25$ ), group 5 or STC30 treated group ( $260.40 \pm 5.75$ ), group 6 or liv.52 HB treated group ( $491.00 \pm 15.63$ ) and group 7 or the mix treatment group ( $408.80 \pm 8.54$ ) where all significantly ( $P \leq 0.05$ ) higher that of normal control or group 1 ( $23.44 \pm 2.88$ ) indicating an ongoing inflammation occasion by possible carcinoma. However, the CRP concentration in all treated groups was significantly ( $p \leq 0.05$ ) reduced compared with the positive control which was not treated. From our results, STC30 appeared to be more potent in reducing the CRP concentration which is also a pointer to reversal of possible cause of inflammation which triggered elevated CRP. Other products used for treatment also showed varying capability is ameliorating CRP triggers which in this study was Hepatocellular carcinoma. Conclusively, significant reduction in CRP levels following various treatment may indicate that the natural products are capable of reducing inflammation caused by the induced hepatocellular carcinoma.

### Keyword

Ganoderma lucidum, Astaxanthin, Liv.52 HB, STC30, C-reactive protein, CCL4, Hepatocellular-Carcinoma.

### Introduction

Cancer is the major cause of death in developed countries and the second in developing countries. The most frequent worldwide-diagnosed cancer is primarily liver cancer which represents

approximately 4% of all new cancer cases diagnosed globally [1]. However, among primary liver cancer, hepatocellular carcinoma is by far the most common histological subtype. Notwithstanding the health promotion and disease prevention campaigns, more than half a million new hepatocellular carcinoma cases are reported yearly, being estimated to grow continuously until 2030. Hepatocellular carcinoma occurs most often in people with chronic liver diseases such as cirrhosis caused by hepatitis B and hepatitis C infection. Chronic infections of hepatitis B or C can aid the development of hepatocellular carcinoma by repeatedly causing the body's own immune system to attack the liver cells some of which are infected by the virus, others merely bystanders [1].

The incidence of HCC in the past 20 years has increased tremendously. Hepatocellular carcinoma accounts for approximately 782,000 new cases and 746,000 deaths worldwide each year based on 2012 estimates [2]. Eighty-five percent of cases are in developing countries, the highest incidence rate reported in areas where hepatitis B virus (HBV) is endemic.

*Ganoderma lucidum* or lingzhi (as known in china) is a popular medicinal mushroom that has been used in China for longevity and health promotion since ancient times. Investigations into the anticancer activity of lingzhi have been performed in both in vitro and in vivo studies, supporting its application for cancer management and prevention. The proposed anticancer activity of lingzhi has prompted its usage by cancer patients. It remains debatable as to whether lingzhi is a food supplement for health maintenance or actually a therapeutic “drug” for medical proposes. Thus far there has been no report of human trials using lingzhi as a direct anticancer agent, despite some evidence showing the usage of lingzhi as a potential supplement to cancer patients. The use of herbs such as lingzhi in cancer curing and preventive treatments remain as questionable or unproven method that have not been scientifically evaluated [3]. Previous studies on anticancer activity of lingzhi, from experiments in vitro and animals to humans’ in vivo, merely supported its applicability for cancer treatment and prevention, and the mechanisms of action have not been fully explored [4-8].

Astaxanthin (AX) is a pigment that belongs to the family of the xanthophylls, the oxygenated derivatives of carotenoids whose synthesis in plants derives from lycopene. Astaxanthin is one of the main pigments included in crustacean, salmonids, and other farmed fish feeds. Its main role is to provide the desirable reddish-orange color in these organisms as they do not have access to natural sources of carotenoids. In addition to its effect on color, one of the most important properties of Astaxanthin is its antioxidant properties which has been reported to surpass those of  $\beta$ -carotene or even  $\alpha$ -tocopherol [9].

Due to its outstanding antioxidant activity Astaxanthin has been attributed with extraordinary potential for protecting the organism against a wide range of ailments such as cardiovascular problems, different types of cancer and some diseases of the immunological system. This has stirred great interest in AX and prompted

numerous research studies concerning its potential benefits to humans and animals. It has been reported that Astaxanthin has antioxidant activity, as high as 10 times more than other carotenoids such as zeaxanthin, lutein, canthaxantin, and  $\beta$ -carotene; and 100 times more than  $\alpha$ -tocopherol. Thus, Astaxanthin has been dubbed a “super vitamin E” [9].

Liv.52 HB is an extensively researched product of The Himalaya Drug Company. Various clinical studies such as open clinical studies, randomized controlled studies, meta-analysis, as well as independent investigator initiated clinical studies have reported that Liv.52 is beneficial in various hepatic conditions. Liv.52 HB contains Nut grass (*Cyperus rotundus*) and Umbrellas Edge (*Cyperus scariosus*) which both have anti-inflammatory and hepatoprotective properties. The ingredients in Liv.52 HB suppress hepatitis B surface antigen (HBsAg) and clears the hepatitis B virus (HBV) by reverse transcriptase inhibition. It significantly lowers the overall viral load in chronic hepatitis B infection. The antiperoxidative activity of Liv.52 HB capsule prevents the loss of functional integrity of hepatic membranes. It also promotes hepatocellular regeneration and protects the hepatic parenchyma. Liv.52 HB reverses the oxidative damage of hepatocytes and exerts an overall hepatoprotective action. It also renormalizes liver functions. Liv 52 HB normalizes liver enzyme levels and restores the hepatic glycogen levels.

Super life Total Care 30 (STC30) is a relatively new health supplement that is known to have lots of benefits and uses. It is an efficient treatment and cure for several diseases and medical conditions – both mild and chronic, it is the origin of stem cell in treatment and prevention of diseases. Stem cells are undifferentiated biological cells that can differentiate into specialized cells and can divide (through mitosis) to produce healthier stem cells. Stem cells are mother cells that have the potential to become any type of cell in the body. One of the main characteristics of stem cells is their ability to self-renew or multiply while maintaining the potential to develop into other types of cells. Stem cells can become cells of the heart, kidney, liver, bones, skin, brain, muscle etc. These basic building blocks of life are fast becoming the ultimate repair kit of the future. Stem cells serve as a sort of internal repair system, dividing essentially without limit to replenish other cells as long as the person or animal is still alive. Due to its observed efficacy, STC 30 has been used and recommended in recent times by some doctors and medical experts as a last resort medication when all else fails. It is also being increasingly adopted by many medical personnel as part of treatment regimens for some chronic diseases, one of which is cancer [10].

This research studied the effects of the natural products (Ayurvedic) commonly used in the management of hepatitis on the C - reactive protein (CRP) concentration of animal model with CCl<sub>4</sub> induced Hepatocellular carcinoma.

The high incidence of hepatocellular carcinoma coupled with the need for discovery of natural products for its treatment due to the toxicity synthetic drug can cause brings a need for research on

and documentation on the available natural products which are used to manage the condition. This study provides information on some natural products which have been believed to be effective for management of Hepatocellular carcinoma using C - reactive protein as a marker.

### Experimental Animals

Thirty five (35) Albino rats of the Wistar strain with a weight range of 60-120g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, and Rivers State, Nigeria. They were housed, and cared for, following the standard rules and regulations of The Institute for Laboratory Animal Research (ILAR).

The animals were allowed to acclimatize for a period of 7 days at the Department of Biochemistry, Federal University Otuoke animal house. They were kept in plastic cages with wire mesh covers to aid ventilation. The animals were kept under monitored environmental conditions of temperature ( $28 \pm 2^\circ\text{C}$ ), relative humidity ( $50 \pm 5\%$ ) and 12-hour light/dark cycle. The animal facility was properly ventilated and the animals were placed on commercial rat pellet as feed and water supplied ad libitum throughout the experimental period.

### Experimental Design and Treatment of Animals

Administration of treatment was done twice daily for a period of twenty-one (21) days via orogastric intubation as described in table 1. The experimental design employed comprised 35 wistar rats of albino strain divided into 7 groups of 5 animals each. Group 1 was the normal control group and the animals in this group received only distilled water. Group 2 was the positive control group, it was induced with HCC along with groups 3, 4, 5, 6 and 7 using intraperitoneal injection of CCl<sub>4</sub> constituted (0.5ml of CCl<sub>4</sub> and 0.5ml of olive oil) and the animals in group 2 received distilled water. Animals in Group 3 to 7 which were also induced with HCC were treated with varying natural products as follows; group 3 received *Ganoderma lucidum*, group 4 received Astaxanthin, group 5 received STC 30, group 6 received Liv.52 HB, group 7 received a mixture of *Galadoma*, Astaxanthin, Liv.52 HB and STC 30.

### Collection of Blood Samples

The animals were sacrificed 12 hours after the last treatment, whole blood was collected from the heart via cardiac puncture using a sterile syringe and needle. The blood samples were put into plain tubes and allowed to clot by standing for 2 hours at room temperature then centrifuged at 4000rpm for 10 minutes to separate the serum from the blood cells. Sera from each centrifuged plain tube were collected into another well labelled plain tubes using Pasteur pipettes. The separate sera were then kept frozen in a refrigerator until when needed for the assays. The C-Reactive protein levels of each animal in the groups were analyzed for in this study using randox kit and biochemical auto-analyzers.

### Analysis of Samples

Serum CRP levels were measured by immunoturbidimetric

method using Roche Cardiac C-Reactive Protein High Sensitive (Roche Diagnostics Penzberg, Germany). The particle-enhanced immunoturbidimetric method was used to measure CRP, with the possibility of measuring CRP within the limits of 0.3 – 350 mg/l. This method quantifies C-reactive protein (CRP) by latex-enhanced nephelometry. Particle-enhanced assays are based on the reaction between a soluble analyte and the corresponding antigen or antibody bound to polystyrene particles. For the quantification of CRP, particles consisting of a polystyrene core and a hydrophilic shell are used in order to link anti-CRP antibodies covalently.

A dilute solution of test sample was mixed with latex particles coated with mouse monoclonal anti-CRP antibodies. CRP present in the test sample forms an antigen-antibody complex with the latex particles. Light scattering, measured by a nephelometric procedure after 6 min, is proportional to the concentration of the analyte present in the sample. An automatic blank subtraction was performed. CRP concentrations were calculated by using a calibration curve. Data reduction of the signals was performed by using a storable logit-log function for the calibration curve. The assay was performed on a Behring Nephelometer for quantitative CRP determination.

### Statistical analysis

The analysis was done in triplicate. Mean results of serum CRP levels obtained were subjected to statistical analysis using One-way Anova at  $P < 0.05$  with the aid of Statistical Package for Social Science version 21.

### Result

Results obtained from the experiment after analysis of the animals' blood samples for the concentration of C – Reactive protein showed that CRP levels for animals in the normal control group or group I ( $23.44 \pm 2.88$ ) was significantly ( $p < 0.05$ ) lower than that of groups II ( $584.20 \pm 11.08$ ), III ( $491.40 \pm 26.81$ ), IV ( $378.00 \pm 37.25$ ), V ( $260.40 \pm 5.75$ ), VI ( $491.00 \pm 15.63$ ) and VII ( $408.80 \pm 8.54$ ). Also, CRP levels for the positive control group or group II ( $584.20 \pm 11.08$ ) was significantly ( $p < 0.05$ ) higher than that of groups III ( $491.40 \pm 26.81$ ), IV ( $378.00 \pm 37.25$ ), V ( $260.40 \pm 5.75$ ), VI ( $491.00 \pm 15.63$ ) and VII ( $408.80 \pm 8.54$ ). The CRP concentration of animals in group III ( $491.40 \pm 26.81$ ) which were treated with *G. lucidum* was significantly ( $p < 0.05$ ) higher than those of groups IV ( $378.00 \pm 37.25$ ), V ( $260.40 \pm 5.75$ ) and VII ( $408.80 \pm 8.54$ ). Results obtained for animals treated with Astaxanthin (group IV) showed that CRP concentration ( $378.00 \pm 37.25$ ) of animals in the group was significantly ( $p < 0.05$ ) higher than that of group V ( $260.40 \pm 5.75$ ) and lower than that of group VI ( $491.00 \pm 15.63$ ). CRP levels of animals in group V ( $260.40 \pm 5.75$ ) which were treated with STC 30 was significantly ( $p < 0.05$ ) lower compared with those of groups VI ( $491.00 \pm 15.63$ ) and VII ( $408.80 \pm 8.54$ ). Results also showed that CRP level of animals of in group VI ( $491.00 \pm 15.63$ ) which were treated using Liv.52 HB was significantly ( $p < 0.05$ ) higher than that of group VII ( $408.80 \pm 8.54$ ).

**Table 1:** Effect of the natural products on C - reactive protein levels of the albino rats.

Group	CRP (Mean ± SEM)
1(NC)	23.44 ± 2.88
2(PC)	584.20 ± 11.08*
3(GAN)	491.40 ± 26.81 <sup>a</sup>
4(AST)	378.00 ± 37.25 <sup>ab</sup>
5(STC)	260.40 ± 5.75 <sup>abc</sup>
6(LIV)	491.00 ± 15.63 <sup>acd</sup>
7(GASL)	408.80 ± 8.54 <sup>abde</sup>

Key: NC = Normal Control, PC = Positive Control, GAN = Ganoderma lucidum, AST = Astaxanthin, STC = STC 30, LIV = Liv.52 HB, GASL = Ganoderma lucidum + Astaxanthin + STC 30 + Liv.52 HB.

\*= significance in comparison with group 1, a=significance in comparison with group 2

b= significance in comparison with group 3, c= significance in comparison with group 4

d= significance in comparison with group 5, e= significance in comparison with group 6

## Discussion

C – Reactive Protein is a blood marker for acute phase inflammation and it's an independent prognostic factor for many human cancers. Increase in CRP levels in groups with induced HCC can be observed from the results and this biomarker elevation when HCC is present is similarly observed in a study published by Carr et al. (2018), The decrease in CRP levels in comparison with the positive control group observed in group III where Ganoderma lucidum was used for treatment is similar to an observation made in the research of Chen et al. (2020), where G. lucidum was used as treatment caused a significant reduction in CRP levels in comparison with untreated groups.

Results obtained from the experiment showed that animals in group IV which were treated using Astaxanthin had significantly lower CRP concentration compared to the positive control group. This CRP level lowering effect observed in group IV is similarly observed in a research by Spiller, which showed that Astaxanthin is capable of significantly reducing CRP levels in the treated group compared to the placebo group. Another research by Xia et al., [11] also showed that Astaxanthin can cause an observed decrease in CRP levels in groups treated with the natural product. Liv.52 HB was the natural product used as treatment in group V and the results of the analysis of animal blood samples in the group revealed a significant decrease in CRP levels compared to that of animals in the positive control group. Research by Sankar et al., tallies with the results of group V in that it showed that a group of Sprague dawley rats treated with Liv.52 HB had significantly lower CRP levels compared to the positive control group in the study.

The CRP level lowering effect (in comparison with the positive control group) observed in group VI may be attributed to some of the components of STC 30 used for treatment in the group. One of such components namely *Vitis vinifera*, was by the study of

Ghalishoura ni et al., and Asbaghi et al. (2020), [12] to be capable of causing reduction in CRP levels. A study by Bounihi et al., which involved the use of *Malus domestica* (another component of STC 30) showed that animal groups treated with it had significantly lower CRP levels compared to the untreated group. Analysis of blood samples of animals in group VII where the treatment was a combination of *Ganoderma lucidum*, Astaxanthin, Liv.52 HB and STC 30 showed that there was a significant reduction in CRP levels of the animals compared to the positive control group.

This observed effect may be said to be due to the synergistic effect of individual components of the treatment mixture used on the animals, as referenced research works above has shown that these natural products have C – Reactive Protein level reducing capabilities. In general, it was observed that administration of the various naturopathic treatments caused significant reduction in CRP concentrations and this may indicate that the natural products are capable of reducing inflammation caused by the induced hepatocellular carcinoma via the following mechanism; acting as cyclooxygenase inhibitor, platelet aggregation inhibitors, beta-adreno receptor antagonist or as angiotensin converting enzyme inhibitor and as an immuno-modulator.

The reduction in the concentrations of C-RP may have been due to plethora of complex biochemical changes occasion by many antioxidants domicile in the natural products used in this study with anti-inflammatory properties.

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