



Effect of Ganoderma lucidum, Astaxanthin, Liv.52 HB and STC30 on Renal Function Parameters of Animal Models with CCL4 Induced Hepatocellular Carcinoma

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Kidneys are vital organ involved in the elimination of waste and toxins from the body and maintenance of homeostasis among other functions. Following the entrance of pathogen or xenobiotics into the body, the immune system respond and in most instances, this responses are out of proportion due to release of pro-inflammatory cytokines and the hyper response of the immune system may end up attacking certain organs of the body and hence, altering the physio-biochemical role of such organ. This study investigated the effect of some natural products commonly used in the management of hepatitis and it associated biochemical alterations on other key systems of the body. Albino Wistar rats weighing 60 to 120g were used for this study. The animals were divided into seven (7) groups of five (5) animals each. Group 1 served as the normal control and were not induced while animal in group 2 to 7 were all induced for Hepatocellular carcinoma using CCl₄. Group 2 served as positive control and where not treated while animals in

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group 3 to 7 received various forms of naturopathic remedy as treatment; *Ganoderma lucidum*, Astaxanthin, Liv 52 Hb, STC30 and combination of all the remedies respectively. The treatment lasted for a period of three weeks (21 days). Twelve (12) hours after last treatment, the animals were sacrificed under chloroform anesthesia and blood samples collected via cardiac puncture. The blood samples were centrifuged at 4000rpm for 10 minutes to obtain the serum. The serum was separated and used for the estimation of serum electrolytes, urea and creatinine to examine the renal functions. Results of the investigation showed that; the serum urea concentration in group III (6.64 ± 0.27), V (3.88 ± 0.15), VI (3.66 ± 0.12) and VII (3.80 ± 0.22) were significantly ($p \leq 0.05$) decreased compared to group II (7.16 ± 0.18) the positive control. Similar trend was also observed for serum creatinine. Serum electrolytes concentration; Na^+ , K^+ , Cl^- and HCO_3^- recorded slight varying degrees of changes across all treated groups at $p \leq 0.05$ compares to the positive and normal control. From our results, HCC induced animals treated with *Ganoderma lucidum*, STC30, Liv 52 Hb and combined treatment showed varying degree of renal function improvement by improving glomerular filtration rate (GFR). The reduction in GFR can be observed in the positive control group as presented by its many abnormal renal parameters' values which is indicative of renal failure and poor GFR. Administration of these natural products however, caused an observed initiation of renormalization in the values of some of the renal parameters with most of the treated groups having values that are significantly different from those of the positive control group at $p \leq 0.05$ which was not treated after induction of HCC. It may thus be concluded that the treatments used had positive effects on the Glomerular filtration rate which led to improved values of some of the renal parameters in the groups treated with these natural products.

Keywords: *Ganoderma lucidum*, *astaxanthin*; *Liv.52 Hb*; *Stc30*; *renal-functions*; *Ccl4*; *hepatocellular-carcinoma*.

1. INTRODUCTION

Hepatocellular carcinoma (HCC) is the most common type of liver cancer, it develops in hepatocytes and can spread from the liver to other parts of the body such as the pancreas, intestines and stomach. Hepatocellular carcinoma, like any other cancer, develops when the epigenetic alterations and mutations affecting the cellular machinery cause the cell to replicate at a higher rate results in the cell avoiding apoptosis [1]. 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. [2]. 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 [3]. Eighty-five percent of cases are in developing countries, the highest incidence rate reported in areas where hepatitis B virus (HBV) is endemic. Hepatocellular Carcinoma rarely occurs in patients younger than 40 years of age. The peak incidence is approximately 70 years old. Certain drugs or

chemical substances have been reported to be effective against HCC, some of which were used in this study. They are administered to the animals with the aim of studying the effects of the supposed treatments on renal parameters of the rats with induced Hepatocellular Carcinoma.

Astaxanthin (AX) is a pigment that belongs to the family of the xanthophylls, an 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 β -carotene or even α -tocopherol [4]. 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 [5]. 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, canthaxanthin, and β -carotene; and 100 times more than α -tocopherol [6]. Thus, Astaxanthin has been dubbed a “super vitamin E” [4].

Ganoderma lucidum or lingzhi as known in china, a popular medicinal mushroom, 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 treatment 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 remains as questionable or unproven methods that have not been scientifically evaluated [7]. 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 [8,9,10, 11,12].

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 the hepatic glycogen levels. (Organic-lab, 2020).

Superlife 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 & 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 of developing 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 [13].

Hepatocellular carcinoma has been long known to be the most common form of primary liver cancer among adults accounting for more than 781,631 deaths per year according to 2018 estimates [14]. Surgical interventions including liver resection, liver transplantation and percutaneous ablation are regarded as the most effective approach with curative potential for liver cancer but due to extra hepatic metastasis and numerous lesions, only about 20% of liver cancer patients are suitable for surgery. Also, some synthetic drugs usually used for HCC management cause toxicity to patients administered these drugs. There is need for alternative treatment options due to high incidence of this form of liver cancer, hence the prompts of research on natural products capable of being used for HCC management.

The high incidence of Hepatocellular carcinoma coupled with the need for discovery of natural products for treatment due to the toxicity synthetic drugs can cause brings a need for research and development of naturopathic

remedies which can be used to manage the disease. This study provides information on some natural products and pharmaceuticals which have been touted to be effective for management of Hepatocellular carcinoma and therefore works towards a reduction in the rate of incidence of the disease. This research aimed to study the effects of the natural products used in this study on some renal parameters of animal models with Hepatocellular carcinoma which was induced using CCl_4 .

2. METHODOLOGY

2.1 Experimental Animals

Thirty five (35) Albino rats of the Wistar strain with a weight range of 60-160g were obtained from the Animal House of the Department of Biochemistry, University of Port Harcourt, 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.

2.2 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_4 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.

2.3 Collection of Blood Samples for Analysis

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 1 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 assays. The concentrations of renal function parameters of each animal in the groups were analyzed for in this study using randox kit and biochemical auto-analyzers.

2.4 Biochemical Assay

All biochemical assays were carried out using randox kits and biochemical analyzers.

The Renal parameters analyzed for in this experiment were serum concentrations of Urea, Creatinine, Sodium, Potassium, Chlorides, and bicarbonate (HCO_3).

2.5 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.

3. RESULTS

3.1 Sodium

Results from our studies showed that sodium levels of animals in the positive control group or group II (131.80 ± 0.80), group III (128.00 ± 1.22), group IV (126.60 ± 0.68), group VI (128.00 ± 0.71) and group VII (132.00 ± 0.71) was significantly ($p < 0.05$) lower than that of the normal control group or group I (136.60 ± 0.87). Also, sodium levels of animals in groups III (128.00 ± 1.22), IV (126.60 ± 0.68) and VI (128.00 ± 0.71) were significantly ($p < 0.05$) lower compared to that of the positive control group (131.80 ± 0.80)

while sodium values for groups V (138.80±1.07).

compared with that of the positive control group (15.20±0.20).

3.2 Potassium

Potassium levels of animals in the positive control group or group II (4.36±0.09), group III (4.96±0.16), group IV (6.18±0.15), group V (4.36±0.16), group VI (4.86±0.12), group VII (4.22±0.11), and group VIII (6.64±0.11) were significantly ($p<0.05$) higher than that of the normal control group or group I (3.78±0.11). The results of the research also showed that potassium levels of groups III (4.96±0.16), IV (6.18±0.15), VI (4.86±0.12) while groups V (4.36±0.16) and VII (4.22±0.11) potassium levels showed no significant ($p>0.05$) difference when compared with that of the positive control group (4.36±0.09).

3.3 Chlorine

Serum chlorine levels of animals in groups V (109.40±1.08) was significantly ($p<0.05$) higher than that of the normal control group or group I (104.40±1.21) while the chlorine levels of animals in group IV (99.20±0.58) was significantly ($p<0.05$) lower than that of the normal control group or group I (104.40±1.21). However, chlorine levels for animals in groups II (103.80±0.86), III (102.80±1.07), VI (102.00±0.71) and VII (106.80±1.16) showed no significant ($p>0.05$) difference when compared to the normal control group (104.40±1.21). The results of the experiment also showed that chlorine levels of animals in group IV (99.20±0.58) was significantly ($p<0.05$) lower than that of the positive control group or group II (103.80±0.86) but those of groups V (109.40±1.08), VII (106.80±1.16).

3.4 Bicarbonate

Results obtained from the analysis showed that bicarbonate concentrations of animals in groups II or positive control group (15.20±0.20), group III (13.80±0.58), IV (10.40±0.24), V (15.60±0.40), VII (15.60±0.51) were significantly ($p<0.05$) lower compared to that of the normal control or group I (17.40±0.40). The bicarbonate levels of animals in groups IV (10.40±0.24) was significantly ($p<0.05$) lower than those of the positive control group (15.20±0.20) but there was no significant ($p>0.05$) difference when bicarbonate levels of groups III (13.80±0.58), V (15.60±0.40), VI (16.20±0.73), and VII (15.60±0.51) were

3.5 Urea

Results obtained after analysis of the animals' blood samples for urea concentrations showed that serum urea levels for animals in groups II or the positive control group (7.16±0.18), III (6.64±0.27), IV (11.86±0.20), V (3.88±0.15), VI (3.66±0.12) and VII (3.80±0.22) were significantly ($p<0.05$) higher compared with that of group I or the normal control group (2.66±0.14). More so, serum urea levels of animals of groups V (3.88±0.15), VI (3.66±0.12), VII (3.80±0.22) were significantly ($p<0.05$) lower than that of the positive control group (7.16±0.18) but that of group IV (11.86±0.20) was significantly ($p<0.05$) higher than the positive control group. There was no significant ($p>0.05$) difference between urea levels for groups II or PC (7.16±0.18) and III (6.64±0.27).

3.6 Creatinine

Results obtained showed that serum creatinine concentrations of animals in the positive control group or group II (85.24±0.34), group III (90.50±1.24), IV (111.42±1.05), V (65.64±0.78), VI (61.06±0.58), VII (59.66±0.83) were all significantly ($p<0.05$) higher than that of the normal control group or group I (53.28±0.49). However, creatinine levels of animals in group III (90.50±1.24) and group IV (111.42±1.05) were significantly ($p<0.05$) higher than that the positive control group (85.24±0.34) while those of groups V (65.64±0.78), VI (61.06±0.58), and VII (59.66±0.83) were significantly ($p<0.05$) lower than that of the positive control group (85.24±0.34).

4. DISCUSSION AND CONCLUSION

Natural products have gained attraction in the pharmaceutical industry as a remedies for plethora of diseases in place of synthetic products due to its relative compatibility with the human body, lesser likelihood of toxicity and effectiveness against various diseases. This study was conducted with the aim of observing the effects of some natural products on renal parameters of animals with hepatocellular damage compared with that of the animals in the normal control and positive control groups which are group I and II respectively. Results from biochemical analysis of blood samples of animals in group III (which were treated using

Ganoderma lucidum) showed that urea levels of animals in the group significantly reduced in comparison with that of the positive control group. A research by Sheena *et al.*, (2003) showed that *G. lucidum* can cause a reduction in serum urea levels in groups treated with the natural product compared to the positive control group and this urea level effect is also observed in the results of our study. The study by Sheena *et al.*, also revealed that *G. lucidum* could significantly reduce serum creatinine levels. This observed effect on serum creatinine levels may have been similarly observed in our study and if higher concentrations of *G. lucidum* was administered to the animal's further reduction would have been possible. In group IV, serum creatinine and urea levels increased significantly in comparison with the PC group and this result tallies with the study by Augusti *et al.*, (2008) which showed that Astaxanthin could not prevent an increase in creatinine levels caused by renal damage. Another study by Nagaraja *et al.*, (2011) showed that treatment of rats with induced HCC using Astaxanthin led to an increase in urea levels in comparison with the positive control group of the experimental setup. Serum urea and creatinine levels for animals in group V was significantly lower than that of the positive control group. This observed effects may be attributed to one of the many components of STC 30 of which one is *Vitis vinifera*. A research published by Abdelsalam *et al.*, (2019) involved the use of *Vitis vinifera* as treatment on Wistar rats and a group where the ethanolic extract of the plant was used showed a significant reduction in serum urea and creatinine levels in comparison with the positive control group. This shows that *Vitis vinifera* could be helpful in renormalizing Glomerular filtration rate thus leading to reduction in serum urea and creatinine levels and also causing significant changes in

levels of electrolytes compared to the positive control group. A research paper by Chowdhury (2020) using Liv.52 HB correlated with the results of this study in that the results of their experiment showed that Liv.52 HB was capable of significantly reducing serum urea and creatinine levels in the treated group compared to the positive control group of the experiment. The results obtained for group VII showed a significant reduction in serum creatinine and urea levels in comparison with the positive control group. This observed effect may simply be as a result of the combined effects of the natural products used as treatment on the Glomerular filtration rate (GFR) of the animals in the group. However, treatment using the natural product combination did not produce any significant difference in serum electrolyte levels of animals in the group (VII) compared to the positive control group. A reduction in GFR causes abnormalities in serum creatinine levels, serum urea levels and electrolyte levels due to the inability of the kidney to selectively absorb some substances and pass out unwanted ones via urine. This reduction in GFR can be observed in the positive control group as presented by it many abnormal renal parameters' values which is indicative of renal failure and poor GFR. Administration of the natural products however, caused an observed beginning of renormalization in the values of some of the renal parameters with many groups having values that are significantly different from those of the positive control group which is in line with work of Marquardt *et al.*, 2015. It may thus be concluded that the treatments used had positive effects on the Glomerular filtration rate which led to better values of some of the renal parameters in the groups treated with these natural products.

Table 1. Experimental grouping and treatment regimen

Experimental group	Treatment regimen	Number of rats
1. Normal control	Distill water intra-gastrically twice a week for 3 weeks	5
2. Positive control (HCC induced and untreated)	Distill water intra-gastrically twice a week for 3 weeks	5
3. Induced and treated	<i>Ganoderma lucidium</i> (12.8mg/kg bw)	5
4. Induced and treated	Treated with 10mg/kg of astaxanthin daily for 2 weeks	5
5. Induced and treated	STC 30 (25mg/kg bw)	5
6. Induced and treated	Liv.52 HB (1.786mg/kg)	5
7. Induced and treated	Galadoma (12.8mg/kg bw), Astaxanthin (10.29mg/kg bw), STC 30 (25 mg/kg bw), Liv.52 HB (1.786mg/kg bw)	5

Table 2. Effect of the natural products on renal parameters of the albino rats

GROUPS	Na (mmol/L)	K (mmol/L)	Cl (mmol/L)	HCO ₃ (mmol/L)	UREA (mmol/L)	CRET (mmol/L)
NC (I)	136.60±0.87	3.78±0.11	104.40±1.21	17.40±0.40	2.66±0.14	53.28±0.49
PC (II)	131.80±0.80*	4.36±0.09*	103.80±0.86	15.20±0.20*	7.16±0.18*	85.24±0.34*
GAN (III)	128.00±1.22 ^{*a}	4.96±0.16 ^{*a}	102.80±1.07	13.80±0.58*	6.64±0.27*	90.50±1.24 ^{*a}
AST (IV)	126.60±0.68 ^{*a}	6.18±0.15 ^{*ab}	99.20±0.58 ^{*ab}	10.40±0.24 ^{*ab}	11.86±0.20 ^{*ab}	111.42±1.05 ^{*ab}
STC (V)	138.80±1.07 ^{abc}	4.36±0.16 ^{*bc}	109.40±1.08 ^{*abc}	15.60±0.40 ^{*bc}	3.88±0.15 ^{*abc}	65.64±0.78 ^{*abc}
LIV (VI)	128.00±0.71 ^{*ad}	4.86±0.12 ^{*acd}	102.00±0.71 ^d	16.20±0.73 ^{bc}	3.66±0.12 ^{*abc}	61.06±0.58 ^{*abcd}
GASL (VII)	132.00±0.71 ^{*bcde}	4.22±0.11 ^{*bce}	106.80±1.16 ^{abce}	15.60±0.51 ^{*bc}	3.80±0.22 ^{*abc}	59.66±0.83 ^{*abcd}

Values are expressed as: Mean ± standard error of mean (SEM), n=5

* = significant at p<0.05 compared with group 1: (NC) , a = significant at p<0.05 compared with group 2: (PC), b = significant at p<0.05 compared with group 3: (GAN), c = significant at p<0.05 compared with group 4: (AST), d = significant at p<0.05 compared with group 5: (STC) e = significant at p<0.05 compared with group 6: (LIV), f = significant at p<0.05 compared with group 7: (GASL)

Key: NC = Normal Control, PC = Positive Control, Na = Sodium, K = Potassium, Cl = Chloride, HCO₃ = Bicarbonate, UREA = Urea, CRET = Creatinine, GAN = Ganoderma lucidum, AST = Astaxanthin, STC = STC 30, LIV = Liv.52 HB, GASL = Ganoderma lucidum + Astaxanthin + STC 30 + Liv.52 HB

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval for this research was obtained from the Research and Quality Directorate of my institution.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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